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Adjuvantibus

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ON THE 60TH BIRTHDAY OF PROFESSOR DR. OTTÓ FEHÉR

OTTÓ FEHÉR was born in Debrecen in 1927. He was educated in the Protestant College of Debrecen and took his medical diploma at the University Medical School. As a student he joined the Department of Physiology headed at that time by Professor ISTVÁN WENT and became involved in the experimental work about regulation of heart and blood circulation. After having finished his studies in 1951, he began postgraduate course in physiology of the nervous system. In examining the problems of synaptic transmission in the symphatetic ganglia he proved that in the postsynaptic membrane of ganglion cells there are two sorts of acetylcholine receptors: a nicotinic type and a muscarinic type, differing in their physiological role and pharmacological responsiveness. This discovery has been confirmed in the international literature repeatedly and gave starting point of the modern theory of neural membrane receptors. It was subsequently proved that denervation and decentralization supersensitivity can be ascribed to redistribution of the two kinds of receptors in the post-synaptic membrane. In these studies he was aided by his first co-worker and friend EMIL BOKRI and students being active in scientific student society: SÁNDOR DAMJANOVICH, PÉTER HALÁSZ, FERENC MECHLER, ELEMÉR LÁBOS,

TIBOR SZABÓ, GYULA MÓZSIK. He defended his candidate thesis in 1960 entitled: The role of acetylcholine-cholinesterase system in the ganglionic transmission of impulses. As a young research worker he introduced first recording of action potentials from peripheral nervous structures.

Beginning with 1962 his attention turned to the physiology of the central nervous system, the cerebral cortex. In collaboration with PÉTER HALÁSZ, FERENC MECHLER made relevant observations on the origin of cortical convulsive potentials and their relation to the sensory evoked potentials. Meanwhile they introduced the method of micro-electrode recording from the cerebral cortex and proved that rhytmic seizure potentials are generated in intracortical reverberation circuits. With Mrs. G. KLITINA, ANTAL BORSOS and ANDRÁS SZABÓ made original observations on Leão's spreading depression.

In 1967 he was invited to be head of Department of Zoophysiology being founded at the József Attila University, that time. Here he initiated an interdisciplinary research to elucidate correlations between electrophysiological signs of synaptic transmission and morphological changes in synaptic ultrastructure. In this work he was aided by ÁRPÁD PÁRDUTZ, FERENC JOÓ and NORBERT HALÁSZ. The interdisciplinary character of the research was supported by the instrumental and conceptual heredity of Professor AMBRUS ÁBRAHÁM, previous head of the Department of Zoology. From the beginning he made efforts to organize the training of students in comparative physiology and offer facilities for practical courses. In this work he was assisted throughout by his first colleagues and friends at the University, GÉZA TURY and LAJOS ERDÉLYI. Beginning with the seventies a new trend of interdisciplinary work started with IMRE ROJIK. He has been studying the fate of labelled amino-acids in the cerebral cortex and introduced a new method for visualizing active nervous structures making use of autoradiography. As the head of the electronmicroscopic laboratory he gave invaluable help to the complex research activity not only for the Department but for the Faculty.

OTTÓ FEHÉR defended his doctoral thesis in 1973 with the title „The origin of cortical evoked and convulsive potentials".

Beginning with 1974 he became engaged in the investigation of heterosynaptic facilitation which is considered to be one of the basic mechanisms underlying formation of memory traces. The exceptional skill and gift of his young co-worker ATTILA BARANYI opened the way to discoveries highly appreciated in the international literature. A new approach to the study of the cortical function was initiated with JOSEPH TOLDI from 1976. The examination of the interactions among cortical evoked potentials in the association areas of cat and rat revealed new dimensions of the neocortical activity. The involvement of MAGDOLNA SZENTE and FERENC PONGRÁCZ in the investigation of central epileptogenic phenomena gave a new surge of this work and brought not only significant results but facilitated the introduction of computer aided evaluation and modelling of electrophysiological processes. Some brilliant experiments of ATTILA BARANYI and the exceptional scientific intelligence of TIBOR GYIMÓTHY helped him to formulate a computer model of epileptic membrane which gives account for most of the cellular manifesta-

tions of this disease. This, and the experiments performed with LAJOS ERDÉLYI and ANDRÁS PAPP on *Helix* neurons served as a basis for discovery of new anti-convulsive substances. These are now pharmacologically and toxicologically tested by HORST SCHULZ. The educational and scientific activity which has been developed at the Department of Comparative Physiology needed continuous technical support by construction and building modern equipments. This was fulfilled by FERENC GYULAI an electric engineer who has been able not only to adopt designs taken from the international literature but has built a lot of new devices based upon his original ideas.

Since 1978 OTTÓ FEHÉR and two co-workers are participating in a common scientific research project together with the Institute of Anatomy of Göttingen headed by professor J.R. WOLFF and with the Neurobiological Group of the Biological Research Institute, headed by FERENC JOÓ and sponsored generously by the Deutsche Forschungsgemeinschaft. The results of this multidisciplinary work about neural plasticity have been published in more than 20 articles.

The Department has a fruitful technical collaboration with the Biological Institute of the Soviet Academy (Puschino) and with the Biological Research Institute of the Yugoslavian Academy (Beograd).

In 1977 OTTÓ FEHÉR was awarded the Academy Prize for his pioneer activity in introduction of new electrophysiological methods in Hungary. Together with Professor GYÖRGY ÁDÁM he is co-author in both editions of the university textbook Comparative Physiology. He is also co-author and editor of the practice book of this discipline.

He has been an active member of the Scientific Qualification Committee, of the Hungarian Physiological Society, of the Hungarian Biological Society, Hungarian Society for Electroencephalography and Clinical Neurophysiology. He was the organizer of several congresses and international symposium. Five of his co-workers have been awarded the degree Candidate of Biological Science and numerous disciples have made his doctoral work under his auspices. With his collaborators he has published 84 scientific articles.

The Department of Comparative Physiology of the József Attila University was founded and developed by professor FEHÉR. He gave a characteristic educational and scientific profile for this new neurobiological school, and brought it in many respects to international level. His progressive attitude to scientific issues and problems of public life guarantee further successes for him and for the Department headed by him.

We wish professor OTTÓ FEHÉR good health and successful activities

the Editorial Board

ACTA BIOL. SZEGED. 33. PP. 7—8 (1987)

PROFESSOR DR. FERENC ZSOLDOS IS 60 YEARS OLD

FERENC ZSOLDOS was born in Sarkad, Hungary, on 24th March, 1927. After secondary school studies in Sarkad and then Békéscsaba, he matriculated in 1947. He next enrolled as a student of biology at the Faculty of Natural Sciences of Eötvös Loránd University, in Budapest, from which he received his diploma in 1952. Following this, he took part in postgraduate training for three years, and then from 1955 worked as an assistant lecturer at the Department of Applied Botany of the University. He moved to the Department of Plant Physiology of the University in Szeged in 1957. He held the position of research worker until 1974, in which year he was appointed Reader, followed in 1984 by his appointment as Professor. He became Chairman of the Department of Plant Physiology in 1985, and continues to hold this position.

FERENC ZSOLDOS started his educational and teaching activities at Eötvös Loránd University where, while still a university student, he regularly took part as a demonstrator in the botany practicals. Later, following his move to Szeged, he led plant physiology practicals, held courses of special lectures, and delivered lectures in the main courses on plant physiology. Under his guidance, an appreciable number of university students have prepared their diploma work and theses for their

degrees, or have obtained their university doctoral degrees. He delivers regular lectures and courses of special lectures on the topics of mineral nutrition, deficiency diseases of plants, and the practical use of hydroponic cultures, within the framework of national (agricultural engineer, teacher, etc.) training courses and postgraduate training courses.

FERENC ZSOLDOS started his research work in 1952 at the Department of Plant Physiology of Eötvös Loránd University, where in 1957 he prepared and successfully defended his university doctoral dissertation, and later his dissertation for the degree of Candidate and Doctor of Science of the Hungarian Academy of Sciences. His scientific activities were greatly influenced by the circumstance that, first as a scholar, and then as a visiting scientist, he was able to spend a long period in 1962 at the Laboratoty of the International Atomic Energy Agency near Vienna (Seibersdorf). Here he learned the modern isotope techniques relating to ion-transport research. Later he started dealing with the effects of environmental stress factors (low temperature, pH, etc.) and various, biologically active compounds (e.g. herbicides) on the mineral uptakes of grain crops. In connection with these topics, he has published 122 papers in national and international scientific periodicals (Physiologia Plantarum, Plant and Soil, Z. Pflanzenphysiologie. etc.) as well as in the publications of international congresses.

FERENC ZSOLDOS is a member of several Hungarian (Szeged Subcommittee of the Hungarian Society of Biologists, Botanical Committee of the Hungarian Academy of Sciences) and foreign (European Society of Nuclear Methods in Agriculture, Scandinavian Society for Plant Physiology, Federation of European Societies of Plant Physiology) scientific societies. He is editor of the journal Botanikai Közlemények (Botanical Communications), and is an editoral board member of Acta Biologica Szegediensis, Physiologia Plantarum and Oryza. He is a member of the advisory board of the "Japan Prize" Selection Committee. The Department of Biological Sciences of the Hungarian Academy of Sciences has commissioned Ferenc Zsoldos to chair the Hungarian National Committee of the Federation of European Societies of Plant Physiology.

Besides his university educational and research activity, FERENC ZSOLDOS takes an active part as project leader in the direction and elaboration of objectives connected with plant mineral nutrition within the main directives of Hungarian agricultural and biological research, in collaboration with Hungarian (Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged; Cereal Research Institute, Szeged; Research Institute for Irrigation; Rice Laboratory, Szarvas) and foreign (Institute of Agriculture, Austrian Research Center, Seibersdorf, Austria) institutions.

As an acknowledgement of his successful activities in university education, research and scientific life, in 1987 he was awarded the honour „For Outstanding Work" by the Hungarian Cultural Minister.

We wish professor FERENC ZSOLDOS good health and successful activities

the Editorial Board.

ACTA BIOL. SZEGED. 33 PP. 9 — 15 (1987)

IAA DISTRIBUTION AND AUXIN SENSITIVITY IN CCC-TREATED BEAN HYPOCOTYLS

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(Received: March 8, 1986)

Abstract

Studies were performed on the distribution of IAA content in the hypocotyls of CCC-treated bean plants, as well as on the sensitivity of the tissues against exogenous IAA. The treatment induces changes in respect to the IAA distribution between the apical and basal parts of the hypocotyls; on the effect of CCC a large part of the IAA content becomes accumulated at the basal part. The elongation growth reaction to exogenous IAA of the hypocotyls of the treated plants does not show any correlation with the endogenous IAA concentration.
Key words: hypocotyl, CCC, IAA-distribution, IAA-sensitivity

Introduction

The growth inhibition occurring as a response to chlorocholine chloride (CCC) treatment is the result of a complex effect, which besides the inhibition of the gibberellin (GA) biosynthesis, also involves the effect exerted on IAA metabolism. In a previous work it was concluded that the amount of diffusible IAA extractable from bean plants shows an increase on the effect of CCC treatment. This indicates ability of the treatment to influence hormone motion within the tissues (NAGY and TABI, 1983), and thus presumedly also the distribution within the organ.

The present paper reports on our studies concerning the IAA distribution and in connection with this, the auxin sensitivity in the hypocotyls of CCC-treated bean plants.

Material and Method

Phaseolus vulgaris L. cv. *Juliska* plants were used for our studies. The seeds were swollen in 500 mg/l concentration of CCC aqueous solution (Merck-Schuchardt) in 25 °C thermostat, then planted in garden mould. The plants were grown under controlled conditions (CONVIRON Cabinet model EF7, equipped with 4x50 W Sylvania incandescent lamps, at 25/20°C day/night temperature, 16 h illumination with 21 Wm^{-2} and 65% relative humidity), and processed at 6 days of age. Measurement of IAA content: the IAA content of 100 hypocotyls was determined after extraction with 80% cold methanol. The extract was evaporated to dryness at lower pressure, then 0,5 M K_2HPO_4 solution was added (pH 9,5). The purifica-

tion and fractionation were carried out according to KAMISAKA and LARSEN (1977). The amount of IAA present in the final acidic ether fraction was measured by the indole-α-pyrone fluorescence method (KNEGT and BRUINSMA, 1973; HEMBERG and TILLBERG, 1980) with Perkin-Elmer spectrofluorometer. All the experiments were carried out in quadruplicates and two parallels. The figures show the mean values of 8 measurements.

IAA solution in 0,001 — 100 mg/l concentration interval was used for measuring the auxin sensitivity of the hypocotyl tissues. The isolated hypocotyls or hypocotyl parts were incubated in light for 24 hours in Petri dishes at 25 °C, under half-sterile conditions.

Results and discussion

EFFECT OF CCC TREATMENT ON THE GROWTH OF THE HYPOCOTYLS

CCC has selective effect on the function of the apical and subapical meristem; thus without affecting the initiative function of the apical meristem leaf and flower, it exerts a strongly inhibiting effect on the subapical meristem function determining the height of the plant (SACHS, 1965; DEEVA, 1980). Since our studies were performed with seedlings, the CCC was introduced into the seed during the course of swelling so as to have it present at the continuation of ontogeny. The applied concentration did not influence the germination of the seeds. The sensitivity of the seedlings against CCC is demonstrated in Fig. 1.

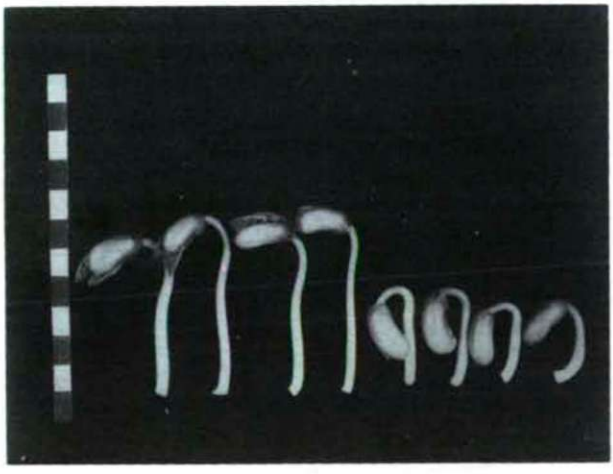

Fig. 1. Effect of CCC in 500 mg/l concentration on the growth of the hypocotyls of *Phaseolus vulgaris* cv. *Juliska* seedlings. Left: control, right: treated.

EFFECT OF CCC TREATMENT ON THE IAA CONTENT AND THE DISTRIBUTION OF THE HYPOCOTYLS

Fig. 2 shows the effect of the treatment on the IAA content of the hypocotyls. The figure also indicates the IAA amount per unit fresh mass, i.e. the IAA concentration.

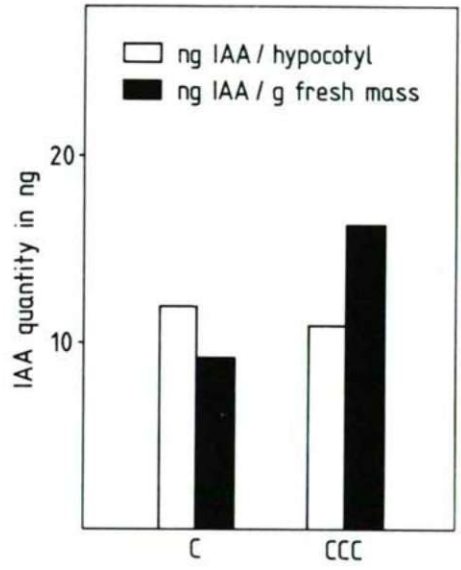

Fig. 2. Effect of CCC on the IAA content extractable by organic solvent.

The IAA content in the hypocotyls of the CCC-treated plants is lower as compared to that of the control. This corresponds to the fact that the total-IAA content in the intact plant decreases on the effect of the treatment compared to the control (NAGY and TABI, 1983), which has also been concluded in case of other plants (KURAISHI and MUIR, 1963; NORRIS, 1966; VOLYNETZ and PALCHENKO, 1977). At the same time, the IAA amount referring to g fresh mass, i.e. the IAA concentration, is higher in the hypocotyl of the treated plant.

Since the IAA distribution within the organ plays important role in the determination of the growth reactions, measurements were also performed in respect to the IAA content in the apical (A) and basal (B) parts of the hypocotyls.

On the effect of the treatment, there is a change in the IAA distribution between the apical and basal parts of the hypocotyls; in the treated plants a large part of the IAA is found accumulated in the basal part of the hypocotyl (Fig. 3). The percental distribution of the total-IAA content concerning the various hypocotyl parts is as follows: *A* in control: 41,46; *B:* 58,54; *A* in treated plants: 21,21; *B:* 78,71%. The higher diffusible amount of IAA extractable from the treated plants (NAGY and TABI, 1983) may be in connection with the higher amount of hormone accumulating in the basal part of the shoot.

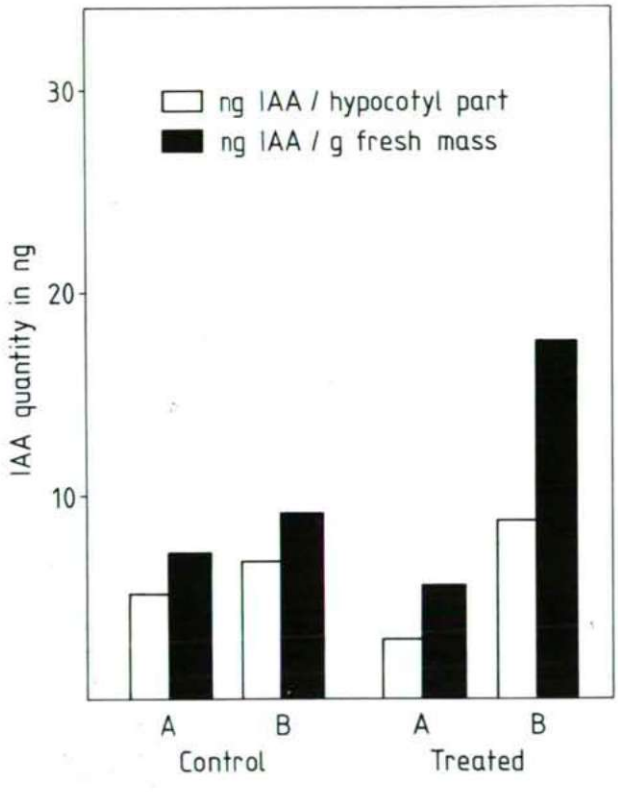

Fig. 3. Effect of CCC treatment on the distribution of the IAA content of the hypocotyls between the apical (A) and basal (B) parts.

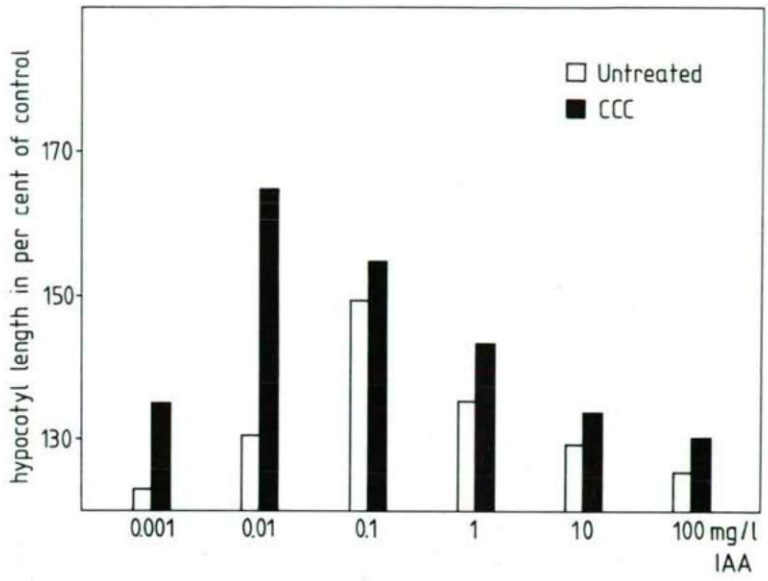

Fig. 4. Effect of CCC treatment on the IAA sensitivity of the hypocotyls.

EFFECT OF CCC TREATMENT ON THE AUXIN SENSITIVITY OF THE HYPOCOTYLS

Studies on the IAA sensitivity of the hypocotyls were performed by means of an indirect method, using exogenous IAA. The growth reactions of the hypocotyls are demonstrated in Figs. 4 and 5. As shown by the results, the hypocotyls of the treated plants react to a lower concentration of IAA than the control, with maximal elongation (Fig. 4). This reaction is as a matter of fact the reaction of the apical (A) part containing the elongation zone (Fig. 5).

As the IAA concentration in the apical part of the hypocotyls of the treated plants is lower than that of the control, assuming receptor molecules of identical number and activity, the saturating dose would be expectable in the higher concentration interval.

The circumstance that in the hypocotyls excised from the treated plants the maximum of the elongation reaction — i.e. the saturating does - is at a lower exogenous IAA concentration, indicates the fact that the elongation reaction is not determined by the absolute degree of the given IAA concentration, but rather by the relationship, ratios of the amount of endogenous growth factors regulating the elongation (in the present case the endogenous IAA and gibberellin). Since CCC

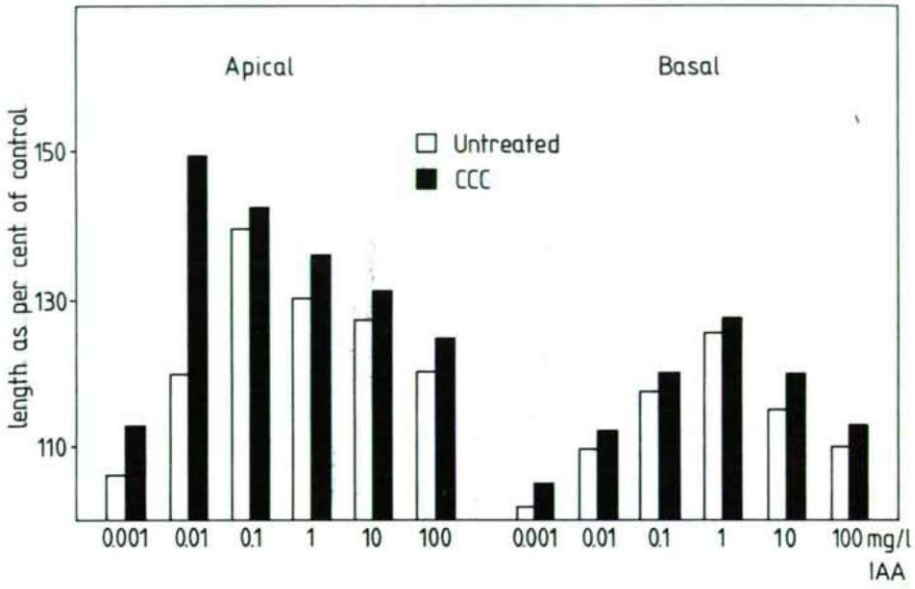

Fig. 5. Effect of CCC treatment on the IAA sensitivity of the apical (A) and basal (B) part of the hypocotyls.

treatment causes significant changes not only in the IAA-. but also in the gibberellin-content and distribution (NAGY, 1986), the developing new hormonal ratios have strong influence on the degree of the elongation reaction of the tissues to the exogenous IAA.

Our results demonstrate that the elongation growth reaction of the hypocotyls of the CCC-treated plants to exogenous IAA does not show any correlation with the endogenous IAA concentration.

References

DEEVA, V.P. (1980): Retardantü — reguljatorü roszta rasztenij. — Nauka i Tehnika, Minszk.

HEMBERG, T. and TILLBERG, E. (1980): The influence of the extraction procedure on yield of indole-3-acetic acid in plant extracts. — Physiol. Plant. *50*, 176-182.

KAMISAKA, S. and LARSEN, P. (1977): Improvement of the indole-α pyrone fluorescence method for quantitative determination of endogenous indole-3-acetic acid in lettuce seedlings. — Plant Cell Physiol. *18*, 595-602.

KNEGT, E. and BRUINSMA, J. (1973): A rapid, sensitive and accurate determination of indolyl-3-acetic acid. — Phytochem. *12*, 753-756.

KURAISHI, S. and MUIR, R.M. (1963): Mode of action of growth retarding chemicals. — Plant Physiol. *38*, 19-24.

NAGY, M. (1987): Klórkolinklorid (CCC) hatása a bab hipokotilok gibberellin-érzékenységére. — Bot. Közl. (in press)

NAGY, M. and TABI, Zs. (1983): Changes in the amount of diffusible auxin and the activity of IAA-oxidase fractions on the effect of 2-chloroethyl-trimethyl-ammonium chloride (CCC). — Acta Biol. Szeged. *29*, 19-22.

NORRIS, R.F. (1966): Effect of CCC on the level of endogenous indole compounds in wheat seedlings. — Can. J. Bot. *44*, 675-684.

SACHS, R.M. (1965): Stem elongation. — Ann. Rev. Plant. Physiol. *16*, 73-96.

VOLYNETZ, A.P. and PALCHENKO, J.A. (1977): Chlorocholine chloride as a possible inhibitor of auxin biosynthesis and a factor limiting its utilisation in growth processes. — Fiziol. Rast. *24*, 1021-1025.

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CHANGES IN PEROXIDASE ACTIVITY AS AFFECTED BY GIBBERELLIC ACID AND CYCOCEL IN CUCUMBER SEEDLINGS

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Abstract

Light and dark grown cucumber seedlings treated with GA_3 and CCC were analysed for growth as well as peroxidase activity in cytoplasmic and salt-extracted ionically wall bound fractions with a view to test the antigibberellin property of CCC. Root elongation was inhibited by both GA and CCC under both the conditions of light and dark, whereas hypocotyl growth was promoted by GA and inhibited by CCC. Peroxidase activity in dark showed inverse relationship with growth promotion. It is suggested that GA induced effect(s) on peroxidase activity is tissue specific and not always of a depressive nature. Further, root peroxidase patterns in GA and CCC indicated that CCC does not always act as an antigibberellin and may have a more direct control on peroxidase synthesis.
Key words: cucumber seedlings, CCC, GA_3, peroxidase activity

Introduction

Cycocel (CCC), a growth retardant has been commonly used to understand the mode of gibberellin action (see SEMBDNER et al., 1980). This is mainly due to the early demonstration (LOCKHART, 1962) that CCC interacts with GA_3 in a specific, competitive manner, and saturating dose of GA_3 can easily overcome the inhibitory effect. CCC inhibits the activity of kaurene synthetase — a key enzyme in GA biosynthesis (FROST and WEST, 1977) and leads to accumulation of geranyl-geranyl pyrophosphate (BARNES et al., 1969). It has also been proposed that CCC acts as an "antigibberellin" (LANG, 1970). However, concentration dependent (REID and CROZIER, 1972) and tissue dependent (GASPAR et al., 1971) controversial reports of CCC cast doubts on its "antigibberellin property". If CCC is a specific antigibberellin it should typically reverse GA action. Although the precise mechanism of GA action is still unknown, it has been proposed that it acts through auxin for elongation growth (PHILLIPS, 1969). Supportingly, GA application resulted in a marked increase in endogenous auxin level (KURAISHI and MUIR, 1963; LAW and HAMILTON, 1984); either by increase in its enzymic syntheis or suppression of its peroxidative breakdown. GA induced inhibition of peroxidase has been shown in a number of cases (FRY, 1979; 1980). Likewise, peroxidase participation in growth regulation and its inverse relation with growth is well documented (see LAMPORT,

1980; LAMPORT and CATT, 1981). Further, that wall bound peroxidase acts as a wall rigidfying factor leading to cessation of elongation growth (FRY, 1979) has also been proposed. It was therefore thought interesting to test the antigibberellin property of CCC using peroxidase activity as a metabolic probe.

Cucumber seedlings were treated with CCC and GA_3 and cytoplasmic and wall bound peroxidase activities were determined during early seedling growth.

Materials and Methods

Seeds of cucumber *(Cucumis sativus* L. Cv. 'Long green'), obtained from Pocha Seeds Company, Poona, India, were surface sterilized for 10 min using 0,1% mercuric chloride ($HgCl_2$). They were then thoroughly washed, rinsed with distilled water and placed over a moistoned filter paper (Whatman 1) in Petri dishes (15 cms). The Petri dishes were kept in a BOD incubator (25 ± 2°C) for 36 h to allow germination. Uniformly germinated seeds were transferred under green safe lamp to other Petri dishes (9 cm ∅) containing a filter paper with 5 ml of (1) Distilled Water (DW), (2) Gibberellic acid (GA_3, 100 mg/l) or, (3) 2-chloroethyl-trimethylammonium-chloride (CCC, 500 mg/l). Two such sets were prepared and incubated in dark and light. Samples were taken at every 24 hourly interval up to 96 h. Growth in terms of length of root and hypocotyl from 20 seedlings was measured and average was taken for calculations of percent over control.

Enzyme extraction

Samples from each treatment were dissected into root and hypocotyl and the organs were chilled for one hour at 0°C. Known amount of organs were homogenized in 5 ml of ice-cold acetate buffer (0.02M, pH 5.0). The homogenate was centrifuged at 12000 g for 10 min and the supernatant served as the source of cytoplasmic peroxidase activity. The pellet was washed with the same buffer till no peroxidase activity could be detected in the washings. The pellet was then incubated for 1 h with 1M sodium chloride to release the ionically wall bound enzyme. It was again centrifuged at 12000 g for 10 min and the supernatant served as wall bound enzyme.

Enzyme assay

The assay method of MAEHLY (1954) was followed and peroxidase activity was measured by recording the change in absorbance at 470 nm (ΔA_{470}) due to the oxidation of guaiacol in presence of H_2O_2. The activity was calculated for ΔA_{470} min^{-1} gm Fr $wt.^{-1}$ and expressed as percentage over DW control values.

Results and discussion

Results presented in Fig. 1 clearly show that in light and dark grown cucumber seedlings GA treatment remarkably inhibited root growth after an initial promotion, whereas hypocotyl growth was significantly promoted. The promotion in hypocotyl growth was highest after 24 and 48 hours of GA application in light and

dark respectively which decreased thereafter. Growth promotion in hypocotyl is accompanied by peroxidase suppression. On the other hand, growth inhibition in roots is accompanied by increase in peroxidase activity. Thus inverse relationships between percent control peroxidase activity and tissue growth is evident; only except in the light exposed hypocotyl. Such inverse relationships between peroxidase activity and growth indicating its active participation in growth reactions are commonly observed. For example reduced growth in dwarf plants is characterized by higher levels of peroxidase activity than the tall plants (SHERTZ et al., 1971). Likewise, while studying peroxidase ontogeny in dwarf pea stems, MCCUNE and GALSTON (1959) reported that GA application to young internodes results in a persistant depression in peroxidase activity and thereby promote the growth of internodes. The physiological significance ascribed to this enzyme is to control the biological activity of IAA in plants (see SEMBDNER et al., 1980) and in fact many of the GA induced growth promotions are explained on the basis of the earlier proposal (PHILLIPS, 1969) that GA affects indirectly via auxin. GA induced increase in auxin levels have been reported in several cases (KURAISHI and MUIR, 1963). Recently, LAW and HAMILTON (1984) using HPLC with electrochemical detection technique confirmed GA induced auxin promotion in dwarf peas.

The absence of a clear inverse relationship between percent control growth and peroxidase activity in light exposed hypocotyls may be due to interference of many other compounds in light. A number of phenolics interfere with peroxidase activity and auxin biosynthesis (KEFELI et al., 1974). According to KEFELI and KUTACEK (1977) phenolic compounds interfere in general, non-specific way with overall metabolic reactions involved in growth processes like photosynthetic and oxidative phosphorylation, biosynthesis of nucleic acids, proteins, etc. Besides phenolics another important factor is the hormonal balance as suggested by LALORAYA et al., (1970) that light causes a switchover from a gibberellin dominant growth and metabolism to the cytokinin-type of responses and that cytokinins do not antagonize gibberellin effects. Thus light triggers a complex set of parameters and they all may be collectively effecting peroxidase levels in hypocotyls. Gibberellins were considered to have little or no effect on root growth (CLELAND, 1969), however, our results show inhibitory effects. Similar results have been reported in a number of cases (SEVNSSON, 1972, LOY and LIU, 1974, BHATT et al., 1976).

Results on CCC induced changes in growth and peroxidase activity in light and dark grown cucumber seedlings are presented in Fig. 2. Like the GA treatment root growth is inhibited at later stages by CCC application but, unlike GA, it also remarkably inhibited the hypocotyl growth under dark and light conditions; the inhibitory effect increased with time. Most of the physiological effect of growth retardants are generally considered to be due to their inhibiting effects on GA biosynthesis (CATHEY 1964, LANG 1970) but our results do not support this conclusion since both GA and CCC inhibit root growth severely at later stages and promote peroxidase activity considerably (Figs. 1, 2). Further, although CCC induced inhibition in growth is accompanied by tremendous increase in peroxidase activity in root and hypocotyl, the trends between peroxidase and growth were

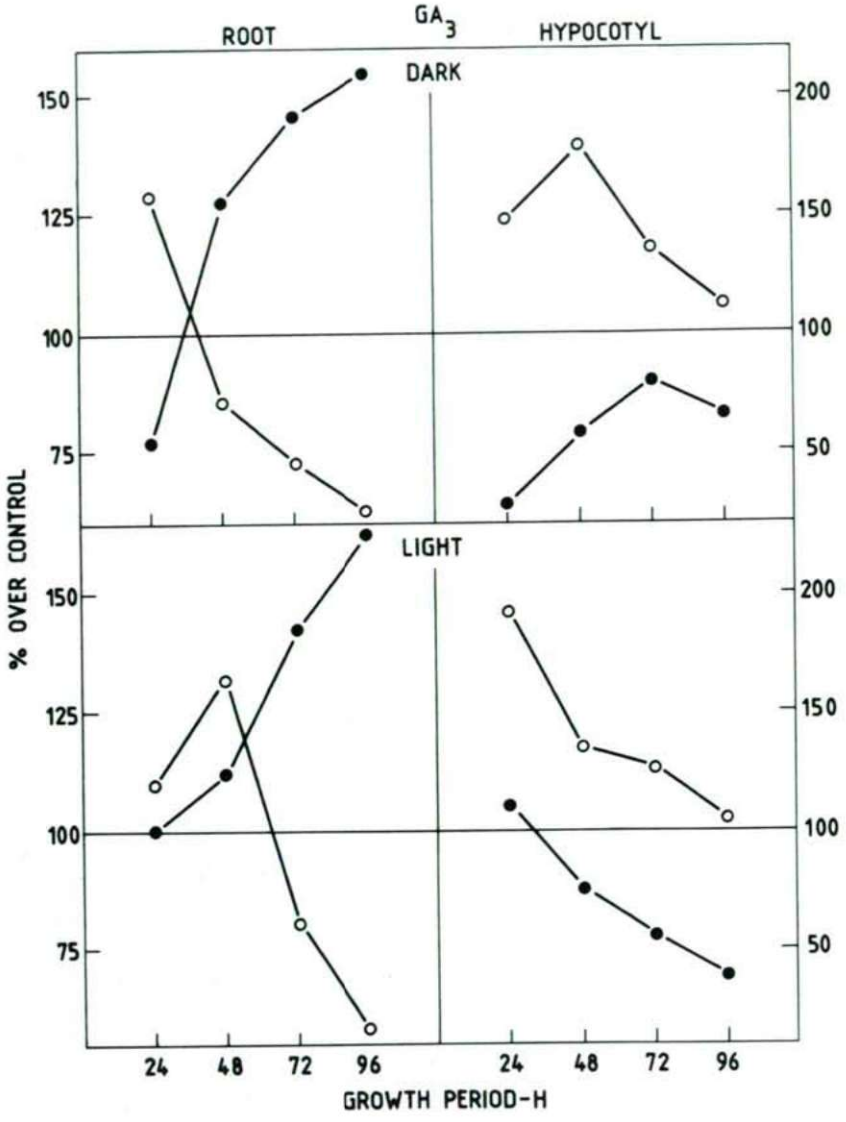

Fig 1. Effect of GA_3 on growth (α) and cytoplasmic peroxidase activity (β) in light and dark grown cucumber seedlings.

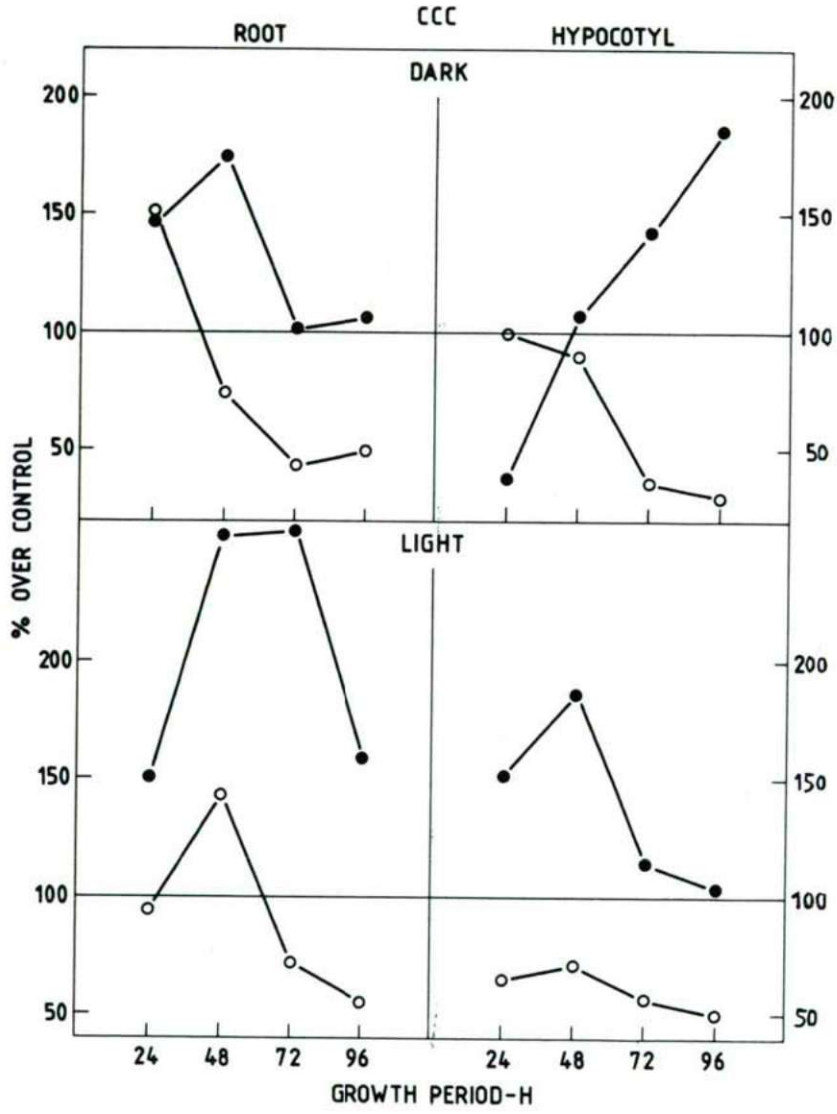

Fig 2. Effect of CCC on growth (α) and cytoplasmic peroxidase activity (β) in light and dark grown cucumber seedlings.

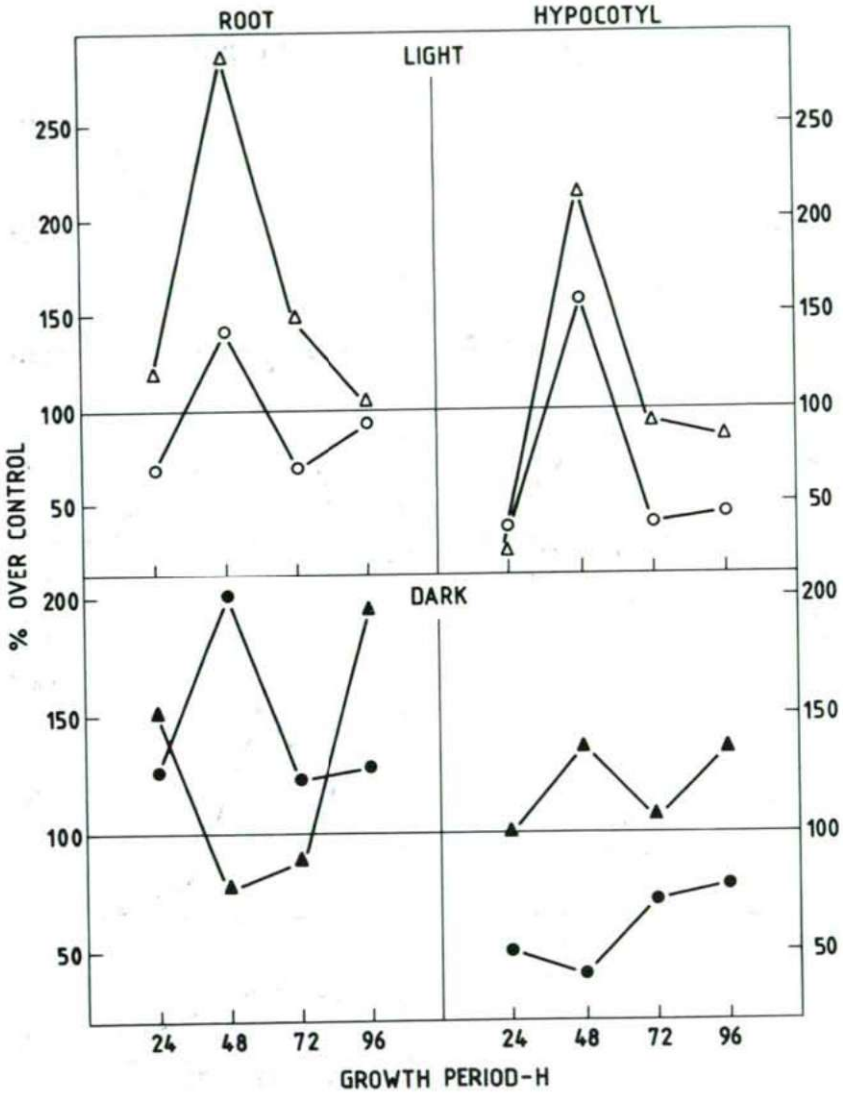

Fig 3. Salt-extractable ionically wall-bound peroxidase activity in GA_1 (α,β) and CCC (ε,ϕ) treated cucumber seedlings; blank and filled symbols represent light and dark respectively.

parallel except in the dark exposed hypocotyl. Thus it appears that CCC acted as antigibberellin only in hypocotyl and not in root. GRAEBE (1968) suggested that only high concentration of growth retardants may reflect an inhibition of GA biosynthesis. In the present work a fairly high concentration (500 mg/l) of CCC was used but even in such situations it did not show antigibberellin activity. Thus CCC may have its own mode of action as suggested CLELAND (1965).

The salt-extracted wall bound peroxidase activity in light grown GA and CCC treated seedlings (Fig 3), showed parallel trends in root as well as hypocotyl. However, under dark conditions GA and CCC possessed inverse trends showing the antigibberellin property of CCC. It is interesting to note that in dark GA promoted wall bound peroxidase in root whereas inhibited it in hypocotyl. Thus GA's effect on peroxidase activity appears to be tissue specific. On the other hand CCC promoted wall bound peroxidase in dark hypocotyl also. Cessation of elongation growth has been well correlated with wall bound peroxidase in diverse systems (RAMA RAO et al., 1982; FRY, 1979).

It is postulated that peroxidase restricts growth in two ways (a) covalently by catalysing the conversion of ferruloyl side chains into diferuloyl cross links and (b) non-covalently by catalysing the conversion of soluble phenolics into hydrophobic biphenyls, polymers or quinones, any of which could protect wall polysaccharides against the attack of wall glucanases.

This work leads us to conclude the following that (I) GA effects on cytoplasmic and wall bound peroxidase are tissue specific, (II) the antigibberellin property of CCC is dependent upon tissue and light conditions, (III) CCC may have its own effects other than GA biosynthesis inhibition, and (IV) peroxidase activity in cytoplasm may be playing an important role in controlling cellular auxin levels while that associated with wall fraction may be responsible for wall rigidification through its action on wall phenolics.

References

BARNES, M., LIGHT, E.N. and LANG, A. (1969): The action of plant growth retardants on trepenoid biosynthesis. — Planta. *88*, 172-182.

BHATT, K.C., VAISHNAV, P.P., SINGH, Y.D. and CHINOY, J.J. (1976): Reversal of gibberellin acid induced inhibition of root growth by manganese. — Biochem. Physiol. Pflanzen. *170*, 453-455.

CATHEY, H.M. (1964): Physiology of growth retarding chemicals. — Ann. Rev. Plant. Physiol. *15*, 271-302.

CLELAND, R. (1965): Evidence on the site of action of growth retardants. — Plant Cell Physiol. *6*, 7-15.

CLELAND, R.E. (1969): The gibberellins. In: Physiology of plant growth and development. ed.: M.B. WILKINS, McGraw-Hill, London, pp. 49-81.

FROST, R.G. and WEST, C.A. (1977): Properties of Kaurene Synthetase from March macrocarpus. — Plant Physiol. *59*, 22-29.

FRY, S.C. (1979): Phenolic components of the primary cell wall and their possible role in the hormonal regulation of growth. — Planta. *146*, 343-352.

FRY, S.C. (1980): Gibberellin-controlled pectinic acid and protein secretion in growing cells. — Phytochemistry. *19*, 735-740.

GASPAR, T., VERBEEK, R. and KHAN, A.A. (1971): Some effects of AMO-1618 on growth, peroxidase and α-amylase which cannot be easily explained by inhibition of gibberellin biosynthesis. — Plant Physiol. *24*, 552-555.

GRAEBE, J.E. (1968): Biosynthesis of Kaurene, Squalene and Phytoene from mevalonate-2-^{14}C in a cell free system from pea fruits. — Phytochemistry *7*, 2003-2020.

KEFELI, V.I. and KUTACEK, M. (1977): Phenolic substances and their possible role in plant growth regulation. In: Plant growth regulation. ed. P.E. PILET, Springer-Verlag, Berlin, Heidelberg, New York, pp. 181-188.

KEFELI, V.I., TURETSKAYA, R. KH. and KUTACEK, M. (1974): In: Hormonal Regulation of plant life, Irkutsk, pp. 35.

KURAISHI, S. and MUIR, R.M. (1963): Mode of action of growth retarding chemicals. — Plant Physiol. *38*, 19-24.

LALORAYA, M.M., SRIVASTAV, H.N., BAKSI, N., SHAH, N., PANDYA, K.J. and SINGH, Y.D. (1972): Role of growth regulators, flavonoid pigments and peroxidase in stomata and trichome differentation. In: Biology of the land plants, eds. V. PURI, Y.S. MURTY, P.K. GUPTA, D. BANERJI, Sarita Prakashan, Meerut (India). pp. 143-159.

LAMPORT, D.T.A. (1980): Structure and function of plant glycoproteins. In: Biochemistry of plants. ed. J. PREISS, Academic Press, New York, *3*, 501-541.

LAMPORT, D.T.A. and CATT, J.W. (1981): Glycoproteins and enzymes of the cell wall. In: Plant Carbohydrates II. Extracellular Carbohydrates. eds. W. TANNER, F.A. LOEWUS, Springer-Verlag, New York, pp. 133-167.

LANG, A. (1970): Gibberellin: Structure and metabolism. — Ann. Rev. Plant. Physiol. *21*, 537-570.

LAW, D.M. and HAMILTON, R.H. (1984): Effects of gibberellic acid on endogenous indole-3-acetic acid and indole-acetyl aspartic acid levels in dwarf pea. — Plant Physiol. *75*, 255-256.

LOCKHART, J.A. (1962): Kinetic studies of certain antigibberellins. — Plant Physiol. *37*, 759-764.

LOY, J.B. and LIU, P.B.W. (1974): Response of seedlings of a dwarf and a normal strain of watermelon to gibberellins. — Plant Physiol. *53*, 325-330.

MAEHLY, A.C. (1954): In: Methods of biochemical analysis. ed. C.D. GLICK, Interscience Publishers. Inc. New York. II, 385-386.

MCCUNE, D.G. and GALSTON, A.W. (1959): Inverse effect of gibberellins on peroxidase activity and growth in dwarf strains of the peas and corn. — Plant Physiol. *34*, 416-418.

PHILLIPS, I.D.J. (1969): Apical dominance. In: Physiology of plant growth and development. ed.: M.B. WILKINS, McGraw-Hill, New York and London, pp. 163-202.

RAMA RAO, N., NAITHANI, S.C., JASDANWALA, R.T. and SINGH, Y.D. (1982): Changes in indoleacetic acid oxidase and peroxidase activities during cotton fibre development. — Z. Pflanzenphysiol. *106*, 157-165.

REID, D.M. and CROZIER, A. (1972): In: Plant Growth Substances. ed. D.J. CARR, Springer-Verlag, Berlin, Heidelberg, New York, pp. 420-427.

SCHERTZ, K.F., SUMPTER, N.A., SARKISSIAN, I.V. and HART, G.E. (1971): Peroxidase regulation by three dwarf height locus in sorghum. — J. Hered. *62*, 235-241.

SEMBDNER, G., GROSS, D., LIEBISCH, H.-W. and SCHNEIDER, G. (1980): Biosynthesis and metabolism of Plant hormones. In: ed.: J. MACMILLAN, Encyclopedia of Plant Physiology, New Series Vol. *9*, Hormonal Regulation of Development I Molecular Aspects of Plant Hormones. Springer-Verlag, Berlin, Heidelberg, New York, pp. 281-444.

SEVNSSON, S.B. (1972): A comparative study of the changes in root growth inducted by cumarin, auxin, ethylene, kinetin and gibberellic acid. — Physiol. Plant. *26*, 115-135.

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CONCENTRATION OF PROLINE IN POLLENS: A NEW TAXONOMICAL INDEX

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Abstract

The free poline of the pollen of 86 plant species have been investigated. It was found that in 66 species the proline content was very high, more than 1.0 per cent of dry matter (proline-type pollens). The proline content of 20 species was lower than 0.3 per cent (non proline-type pollens). The high proline accumulation is not connected with phylogenetical development. Proline-type pollen occurred among gymnosperms as well as among dicotyledonous and monocotyledonous angiosperms. Extent of accumulation of proline in pollen showed no correlation with the mode of pollination; proline-type pollen occurred among entomogameous, anemogameous and autogameous species alike.

Mature pollen contained in general 1.0 — 2.5 per cent proline which could be quickly demonstrated with our method based on a colour reaction with isatin. Proline accumulation is not uniform within families; 6 families were found which had proline-type and non-prolyne-type species as well. Species of the same genera, however, were alike in this respect: they were all of the proline-type or all of the non-proline-type. Therefore the isatin reaction of the pollen could be considered as a chemotaxonomical index.

Key words: Angiosperms, Dicotyledons, Gymnosperms, Istain reaction, *Monocotyledons,* Pollens.

Introduction

It is already known that free proline accumulates in extreme high concentrations in the pollen of many species; its concentration is higher than that of the other protein amino acids altogether (TUPÝ, 1963; AHOKAS, 1978; HESLOP-HARRISON, 1979; DASHEK and MILLS, 1981; PÁLFI et al. 1981).

DASHEK and HARWOOD (1974), RAI and STOSKOPF (1974), MASCARENHAS (1975), ALARKON et al. (1978), DASHEK and MILLS (1980) established that proline has an important role in the energetic transformations of the pollens interacting with the stigma. At the same time proline is an effective activator of the Krebs cycle and it regulates the water balance and the function of several enzymes. Other authors also publicated that proline has an important role in the synthesis of the glycoproteins rich in hydroxy-proline which glycoproteins are necessary for the elongation of the cell walls and pollen tubes (TUPÝ, 1963; DASHEK et al. 1971; BRITIKOV, 1975; KURSAKOV and RYZHKOV, 1980; DASHEK and MILLS, 1981).

Proline increases vitality through its positive effect on drought-resistance and cold-resistance as well (PÁLFI and JUHÁSZ 1970; YAMADA and KONO 1976; TYANKOVA, 1980; TYMMS and GAFF, 1979; LEWITT, 1980; PALEG and ASPINALL, 1981; SIMINOVITCH and CLOUTIER, 1981; THEBUD and SANTARIUS 1981; van SWAAIJ and JAKOBSEN, 1985).

In this paper authors tried to answer the question: which proline concentrations occur in the mature pollen grains by the most species.

Investigating the pollen of many species authors tried to establish the occurrence of high proline accumulation among the plants, whether it occurs in the more ancient families or in the more advanced ones. The most important task was to elucidate to a certain degree whether high proline accumulation could be used as a chemotaxonomical index.

PÁLFI (1982) and PÁLFI and KÖVES (1984) already published a new rapid staining method with isatin with the aid of which proline content of individual pollen grains can be determined. In this paper proline concentrations of the extracts of pollens are compared with per cents of positivity of the new isatin staining reaction.

Materials and Methods

Names of families and species investigated can be seen in the Tables. The pollen were fixed and dried at 90 °C on the day of harvesting. After this kind of conservation the staining with isatin can be performed immediately or even after 1 or 2 years; proline concentration of the fixed pollen does not change (EPPENDORFER and RILLE 1973; PÁLFI et al. 1974).

The new formule of our isatin reagent is as follows: to 20 ml acetone 0.4 ml glacial acetic acid is added and in the mixture 0.20 g isatin is solved. The staining is performed on slides detailed description of the method is already published (PÁLFI 1982; PÁLFI and KÖVES, 1984; PÁLFI and GULYÁS 1985). For the evaluation of the staining light microscope was used, magnification 100 — 300 x according to the size of the pollens. Proline content of the amino acid extracts was determined according to ASPINALL et al. (1973) in 4 repetitions; the mean values are here reported. In cases where the deviation of individual values from the mean was more than ± 5 per cent the measurings were repeated.

Result and discussion

Mature pollen grains were stained with the isatin reagent intensive blue, dark blue or black due to their high proline content; this is the positive isatin reaction. Pollen with low proline concentration retain their original colour or they are stained light brown (negative isatin reaction). On the black and white microphotos pollen with high proline content are black while that of low proline content show different shades of grey (Plate I. and II.). On the photos occur mostly black and grey pollens (containing much and few proline respectively) together. The photos show only a part of the field of sight. Mostly such fields were counted on which 120 — 150 pollen grains could be seen, the staining values of 5 fields were averaged and the per cent of

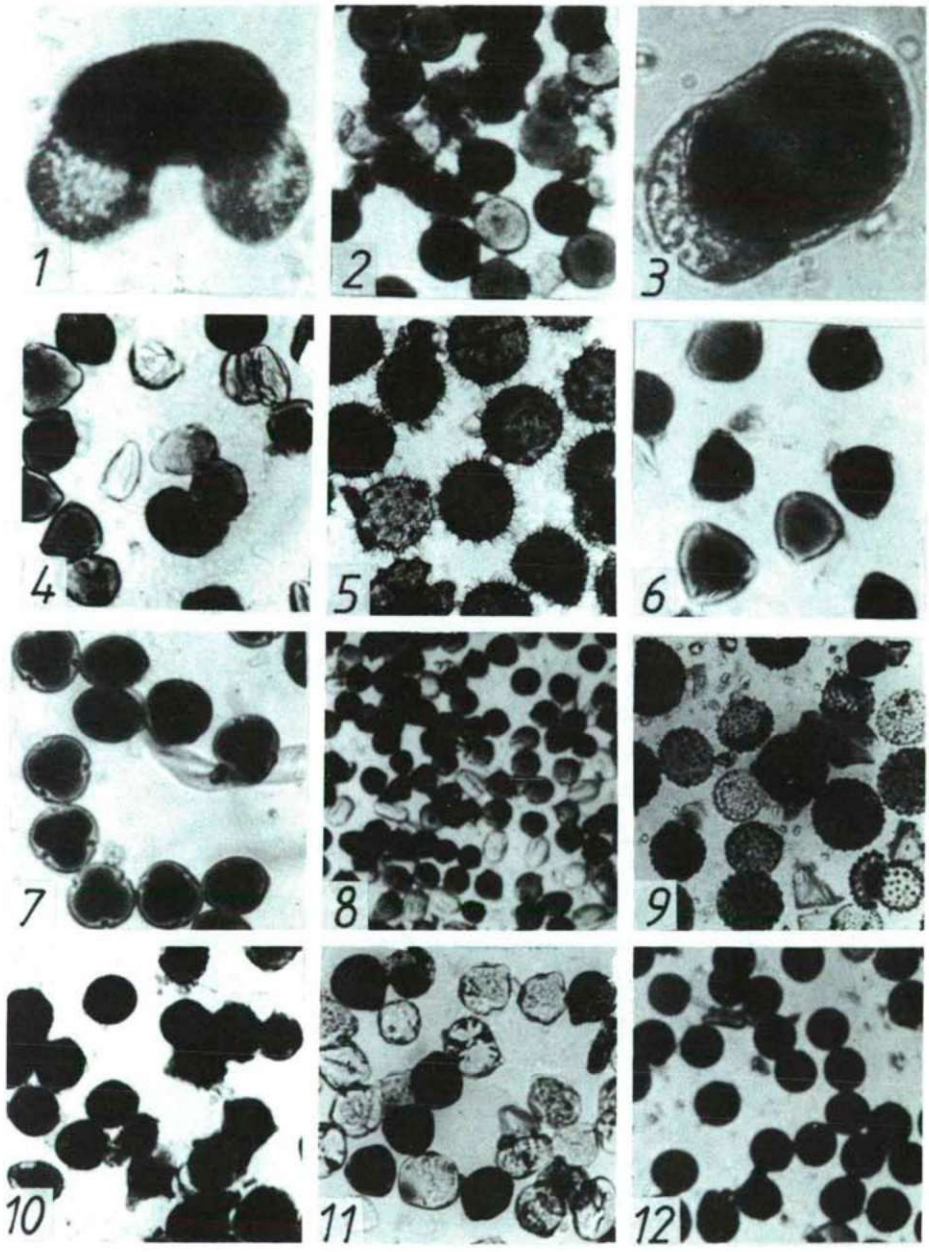

Plate 1. Pollen grains of many species stain dark blue or black (on the photo black) with the isatin reagent due to their high proline concentration. The grey pollens contain very few proline. The magnification is different at the different species; generally 100 — 200 x, at *Pinus* 600 x. 1 = *Pinus nigra;* 2 = *Ranunculus acer;* 3 = *Pinus silvestris;* 4 = *Malus pumila;* 5 = *Hibiscus rosa-sinensis;* 6 = *Armeniaca vulgaris;* 7 = *Tilia cordata;* 8 = *Rosa canina;* 9 = *Abutilon theophrasti;* 10 = *Solanum melongena;* 11 = *Solanum tuberosum;* 12 = *Datura inoxia.*

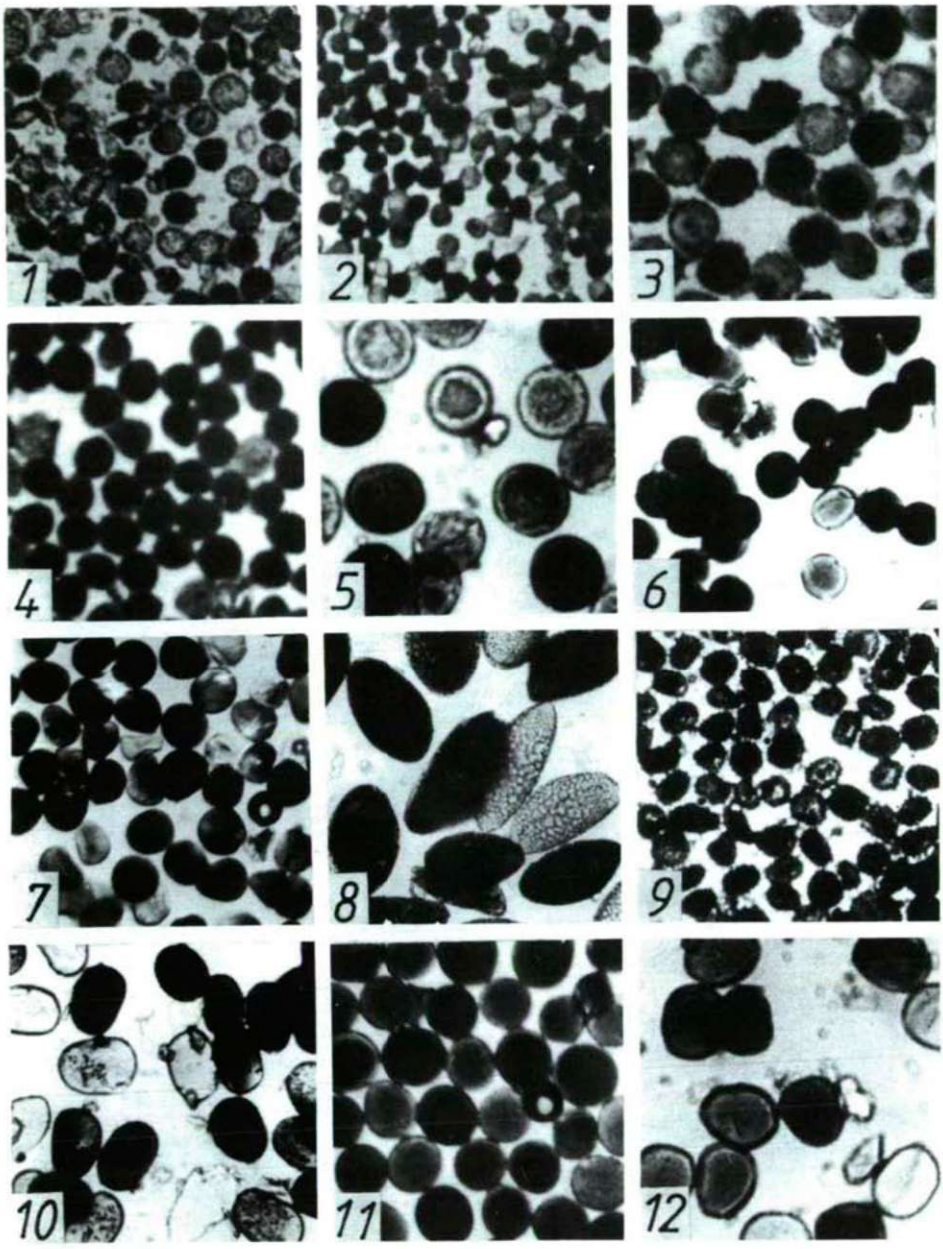

Plate 2. Pollen grains stained black are of extremely high proline concentration while the grey ones contain only an insignificant amount of proline. Magnification generally 100 — 200 x; by *Juglans* (5) and *Hemerocallis* (8) 500 x, by *Secale* 300 x.
1 = *Dahlia variabilis;* 2 = *Papaver rhoeas;* 3 = *Chrysanthemum hortorum;* 4 = *Corylus avellana;* 5 = *Juglans regia;* 6 = *Salix babylonica;* 7 = *Triticum aestivum;* 8 = *Hemerocallis fulva;* 9 = *Colchicum autumnale;* 10 = *Secale cereale;* 11 = *Zea mays;* 12 = *Hordeum vulgare.*

positive staining calculated. Besides valuating the colours of the isatin reaction the proline concentration of the pollen extracts was also reported (Table 1.).

Serial numbers of the 45 species in Table 1. approximately correspond to their state of development i.e. to their phylogenetic sequence. The species belong to 20 families. In Table 1. entomogameous, anemogameous and autogameous species together occur. It can be seen that proline concentration of the pollen extracts was higher than 1.0 per cent by all of the 45 species and even higher than 2.0 per cent by 9 species. Per cents of the pollen grains showing positive isatin reaction varied between 30 and 95.

Table 1. shows that the proline concentration of the pollen extracts is directly and proportionally correlated with the per cents of the positive isatin reaction.

Table 2. shows results about the most developed plants: the monocotyledons.

In Table 2. data of proline concentration and of positive isatin reaction of 16 entomogameous, anemogameous and autogameous species belonging to 3 families are reported. Proline concentration of the pollen extracts varies between 1.11 and 2.38 per cent. It can be seen that the lowest per cent of isatin reaction was given by the species which showed the lowest proline concentration (No. 7.: *Lilium regale*). And the species having the highest proline concentration shows the highest per cent of positive isatin reaction (No. 16: *Zea mays*). There are no essential differences between the dicotyledonous and the monocotyledonous species although they represent two entirely different levels of development.

In Table 3. species are reported in which proline concentration of the mature disseminating pollens is entirely different as compared with the afore-mentioned species. Data of 16 species belonging to 10 families (dicotyledons and monocotyledons together) are reported here. The proline concentration of their pollen extracts is very low: 0.12 — 0.28 per cent of dry matter. At such a low proline concentration the isation reaction is negative: no dark blue or black staining of pollen grains occur. In the case of these 16 species our isatin reaction unsuitable for the determination of proline concentration. Among these species there occur species of the lower and higher level of phylogenesis as well.

Data based on the proline concentration reported here and in earlier papers (PÁLFI 1982; PÁLFI and KÖVES, 1984) a concentration value was sought with the aid of which the species could be divided into two groups: 1. proline-type" species where proline concentration of the mature pollens reaches 1.0 per cent of dry matter and 2. non-proline-type" species where proline concentration in the mature pollens is much more lower than 1.0 per cent (mostly ten times lower than at the species of the first group).

Pollens of the most inferior flowering plants, the gymnosperms were also investigated (Table 4.).

As reported in Table 4. among the 9 species belonging to 3 families there are 5 of the proline type and 4 of the non-proline-type. Accordingly, these 2 types of proline accumulation occurred already in the most ancient flowering plants: the grade of proline accumulation does not indicate neither a primitive nor an advanced state.

Families	Species	Proline concentration of the extracts	Positive reaction with isatin
		percent	
Ranunculaceae	1. *Ranunculus acer*	1.54	62
Nymphaeaceae	2. *Nymphaea alba*	1.29	45
Rosaceae	3. *Rosa canina*	2.23	91
	4. *R. polyantha*	1.87	81
	5. *Pyrus communis*	1.28	44
	6. *P. achras*	1.24	43
	7. *Malus pumila*	1.38	54
	8. *M. floribunda*	1.15	35
Grossulariaceae	9. *Ribes aureum*	1.37	56
	10. *R. rubrum*	1.19	38
Fabaceae	11. *Trifolium repens*	1.36	52
	12. *T. pratense*	1.17	38
	13. *Robinia pseudo-acacia*	1.47	53
	14. *R. hispida*	1.36	52
Eleagnaceae	15. *Elaeagnus angustifolia*	1.56	64
Hippocastanaceae	16. *Aesculus hippocastanum*	1.82	80
Caprifoliaceae	17. *Sambucus nigra*	1.18	35
Tiliaceae	18. *Tilia cordata*	2.10	88
	19. *T. platyphyllos*	2.22	94
Malvaceae	20. *Abutilon theophrasti*	1.41	54
	21. *Hibiscus rosa-sinensis*	1.12	33
Solanaceae	22. *Solanum tuberosum*	1.37	53
	23. *S. melongena*	1.54	62
	24. *S. nigrum*	1.50	59
	25. *Datura inoxia*	1.68	69
	26. *D. arborea*	1.57	63
Papaveraceae	27. *Papaver somniferum*	1.46	56
	28. *P. rhoeas*	2.37	93
Cruciferae	29. *Lepidium draba*	1.36	55
Cucurbitaceae	30. *Cucumis sativus*	1.42	53
Compositae	31. *Dahlia variabilis*	1.46	57
	32. *Chrysanthemum hortorum*	1.42	50
	33. *C. corymbosum*	1.39	47
	34. *C. leucanthemum*	1.56	64
Primulaceae	35. *Primula veris*	2.20	92
	36. *P. acaulis*	2.16	90
Betulaceae	37. *Corylus avellana*	2.24	95
	38. *Betule pendula*	1.08	30
Fagaceae	39. *Quercus robur*	1.59	63
Juglandaceae	40. *Juglans regia*	1.10	30
Salicaceae	41. *Populus tremula*	1.12	31
	42. *P. alba*	1.26	45
	43. *Salix babylonica*	2.18	91
	44. *S. cinerea*	1.69	68
	45. *S. smithiana*	2.05	87

(Average deviation being below ± 5 per cent; n = 4 and 5)

Table 1. Proline concentration in per cent of dry matter in pollen extracts of dicotyledonus species and per cent of positive isatin reaction of the pollen grains. Among the 52 species belonging to 20 families there are entomogameous,anemogameous and autogameous species. Pollens of the proline-type".

Table 2. Proline concentration in pollen extracts and positive isatin reaction of the pollen grains of monocotyledonous species. Pollens of the proline-type".

Families	Species	Proline concentration of the extracts	Positive reaction with isatin
		percent	
Liliaceae	1. *Colchicum autumnale*	2.18	89
	2. *Hemerocallis fulva*	1.39	52
	3. *Allium schoenoprasum*	1.42	55
	4. *Allium cepa*	1.88	80
	5. *Lilium candidum*	1.14	41
	6. *L. martagon*	1.27	50
	7. *L. regale*	1.11	39
	8. *Muscari comosum*	1.63	71
Iridaceae	9. *Iris pumila*	1.26	45
	10. *I. germanica*	1.32	50
Gramineae	11. *Secale cereale*	1.17	38
	12. *Triticum aestivum*	1.51	59
	13. *T. durum*	1.47	54
	14. *Hordeum vulgare*	1.67	73
	15. *Oryza sativa*	1.93	82
	16. *Zea mays*	2.38	92

Evaluating the data of the Tables 1. — 4. it turns out that from the 86 species investigated 66 are of the proline-type and 20 species of the non-proline-type — according to authors' data covering species from 30 families.

Generally 2.0 — 2.5 per cent proline concentration is the highest.

These data also show that the extent of proline accumulation has no correlation with the family boundaries. Six families were demonstrated in which proline-type pollens as well as non-proline-type pollens occurred *(Fabaceae, Malvaceae, Cruciferae, Cucurbitaceae, Compositae, Liliaceae)*.

In contrast to this, investigated species belonging to the same genus were all of the proline-type or all the non-proline-type. Therefore, when species assigned to the same genus would be different as regards their proline type, a revaluation of their taxonomical situation is indicated — proline type being a genus charasteristic.

Following genera are of the same proline type: by the gymnosperms (Table 4.) all of the 5 *Pinus* species are of the proline-type. At the same time the 3 *Juniperus* species are of the non-proline-type. In the angiospermous dicotyledons (Table 1.) all investigated species of the genera *Rosa, Pyrus, Malus, Trifolium, Robinia, Tilia,*

Solanum etc. are of the proline-type.In the most advanced class (monocotyledons; Table 2.) there are also genera the species of which are all of the proline-type *(Allium, Iris, Lilium)*. In the table of the non-proline-type species (Table 3.) there are also species belonging to the same genus (the dicotyledonous *Cucurbita* and the monocotyledonous *Tulipa*). Consequently, the isatin reaction of the pollens could be properly used for the chemotaxonomic characterization of genera.

Table 3. Dicotyledonous and monocotyledonous species having pollen with such a low concentration of proline that they are not stained with the isatin reagent. Pollens of non-proline-type"

Families	Species	Proline concentration of the extracts	Positive reaction with isatin
		percent	
Fabaceae	1. *Coronilla varia*	0.20	—
Malvaceae	2. *Lavathera thuringiaca*	0.27	—
Labiatae	3. *Ajuga genevensis*	0.28	—
	4. *Lamium purpureum*	0.22	—
	5. *Glechoma hederacum*	0.18	—
Cruciferae	6. *Brassica napus*	0.26	—
	7. *Arabis procurrens*	0.15	—
Tamaricaceae	8. *Tamarix tetranda*	0.12	—
Begoniaceae	9. *Begonia semperflorens*	0.23	—
Cucurbitaceae	10. *Cucurbita pepo*	0.17	—
	11. *C. ficifolia*	0.21	—
	12. *C. maxima*	0.20	—
Compositae	13. *Helianthus annuus*	0.23	—
Liliaceae	14. *Tulipa germanica* (gelb)	0.18	—
	15. *T. gesneriana* (rot)	0.23	—
Amaryllidaceae	16. *Narcissus pseudo-narcissus*	*0.28*	—

Among the 86 species in the Tables 1.—4. occur autogameous, entomogameous and anemogameous species; mode of pollination is not connected with the proline concentration of the pollens.

It was demonstrated by KURSAKOV and RYZHKOV (1980) in the case of *Ribes nigrum* and PÁLFI and KÖVES (1984) in the case of *Zea mays* that adding free proline to the substrate of the in vitro germination experiments elongation of the pollen tubes increased for 100 per cent as compared with the control without proline. This indicates that the presence of a great quantity of proline is really advantageous during the germination of the pollens (LEWITT, 1980; PALEG and ASPINALL, 1981; ELTHON and STEWART, 1984). Notwithstanding, all 2 plants species in these experiments are of the proline-type *(Ribes nigrum and Zea mays)*.

Table 4. Proline concentration in pollen extract and isatin reaction of pollen grains of gymnospermous species. Proline concentration in the pollens of 4 species is very low and therefore these do not give positive reaction with isatin.

Families	Species	Proline concentration of the extracts	Positive reaction with isatin
		percent	
Taxaceae	1. *Taxus baccata*	0.06	—
Abietaceae	2. *Pinus silvestris*	1.28	42
	3. *P. nigra*	1.45	58
	4. *P. strobus*	1.17	39
	5. *P. mugo*	1.23	41
	6. *P. griffithii*	1.14	36
Cupressaceae	7. *Juniperus communis*	0.19	—
	8. *J. virginiana*	0.13	—
	9. *J. chinensis*	0.16	—

References

AHOKAS, H. (1978): Cytoplasmic male sterility of barley. II. Physiology and anther cytology of msml. — Hereditas. *89*, 1-21.

ALARKON, N.A., LARIONOVA, E.G. and MININA, T.K. (1978): Amino acid of pollen of *Pinus sibirica*. — Fiziol. Rast. *26*, 186-189.

ASPINALL, D., SINGH, T.N. and PALEG, L.G. (1973): Stress metabolism. V. Abcisic acid and nitrogen metabolism in barley and *Lolium temulentum* L. — Austral. J. Biol. Sci. *26*, 319-327.

BRITIKOV, E.A. (1975): Biologicheskaya rol prolina. — Izd. Nauka". Moskva.

DASHEK, W.V., HARWOOD, H.I. and ROSEN, W.G. (1971): The significance of a wall bound hydroxyproline-rich glycopeptide in lily pollen tube elongation. — In: HESLOP-HARRISON, J. (Ed.) Pollen development and physiology. Butterworths, London.

DASHEK, W.V. and HARWOOD, H.I. (1974): Proline, hydroxyproline and lily pollen tube elongation. — Ann. Bot. *38*, 947-959.

DASHEK, W.V. and MILLS, R.R. (1980): Azetidin-2-carboxylic acid and lily pollen tube elongation. — Ann. Bot. *45*, 1-12.

DASHEK, W.V. and MILLS, R.R. (1981): Proline metabolism by germinating Lilium longiflorum. I. Labelling of cytoplasmic wall and culture medium molecule. — Acta Soc. Bot. Pol. *50*, 51-66.

ELTHON, T.E. and STEWART, C.R. (1984): Effect of proline analog L-Thiazolodine-4-carboxylic acid on proline metabolism. — Plant Physiol. *74*, 213-218.

EPPENDORFER, W.H. and RILLE, S.W. (1973): Decomposition in presence of nitrat of amino acids especially tyrosine, during acid hydrolysis of plant material and standard amino acid solution. — Plant and Soil. *38*, 215-218.

HESLOP-HARRISON, J. (1979): Aspects of the structure, cytochemistry and germination of the pollen of rye *(Secale cereale)*. — Ann. Bot. *44*, Suppl. 1.

KURSAKOV, A.G. and RYZHKOV, S.D. (1980): The content of free amino acids in oppositely charged fractions of black currant pollen. — Fiziol. Raszt. *27*, 735-739.

LEVITT, J. (1980): Responses of plants to environmental stresses. II. Water, radiation, salt and other stresses. — Acad. Press. New York, London, Toronto, Sydney, San Francisco.

MASCARENHAS, J.P. (1975): The biochemistry of angiosperm pollen development. — Ann. Bot. *41*, 259-314.

PALEG, L.G. and ASPINALL, D. (1981): The physiology and biochemistry of drought resistance in plants. — Acad. Press. Sydney, New York, London, Toronto, San Francisco.

PÁLFI, G. and JUHÁSZ, J. (1970): Increase of proline level in water deficient leaves as a reaction to saline or cold root media. — Acta Agron. Acad. Sci. Hung. *19*, 79-88.

PÁLFI, G., KÖVES, E. and BITÓ, M. (1974): The role of amino acids during water stress in species accumulating proline. — Fyton. *32*, 121-127.

PÁLFI, G., PINTÉR, L. and PÁLFI, Zs. (1981): The proline content and fertility of the pollen of inbred maize lines. — Acta Bot. Acad. Sci. Hung. *27*, 179-187.

PÁLFI, G. and PÁLFI, Zs. (1982): A rapid method for the determination of fertility of maize pollen with the proline-isatin reaction. — Maydica. *27*, 107-111.

PÁLFI, G. and KÖVES, E. (1984): Determination of vitality of pollen on the basis of its amino acid content. — Biochem. Physiol. Pflanzen. *179*, 237-240.

PÁLFI, G. and GULYÁS, S. (1986): Complementary data to the vitality test of pollens. — Növénytermelés (Hung. res. eng.). *34*, 351-358.

RAI, R.K. and STOSKOPF, N.C. (1974): Amino acids comparisons in male sterile wheat derived from Triticum timopheevi ZHUK. Cytoplasm and its fertile counterpart. — Theor. Appl. Genet. *44*, 124-128.

SIMINOVITCH, D. and CLOUTIER, Y. (1981): 24-Hour induction of freezing and drought resistance in plumules of winter rye seedlings by desiccation at room temp. in the dark. — Plant Physiol. Suppl. *67*, 345.

THEBUD, R. and SANTARIUS, K.A. (1981): Effect of freezing on isolated plant mitochondria. — Planta. *152*, 424-427.

TUPÝ, J. (1963): Free amino acids in apple pollen from the point of view of its fertility. — Biol. Plantarum. *5*, 154-159.

TYANKOVA, L. (1980): Soderzhanie prolina, svobodnih aminokislot, obshchego i belkovogo azota i sahar kak funkcijah prodolzhitelnosti i stepeni podsuhaniya otdelennih listev tabaka. — Fiziol. Rast. *27*, 806-813.

TYMMS, M.J. and GAFF, D.F. (1979): Proline accumulation during water stress in resurrection plants. — J. Exp. Bot. *30*, 165-168.

VAN SWAAIJ, A.C., JACOBSEN, E. and FEENSTRA, W.J. (1985): Effect of cold hardening, wilting and exogenously applied proline on leaf proline content and frost tolerance of several genotypes of *Solanum*. — Physiol. Plant. *64*, 230-236.

YAMADA, N. and KONO, Y. (1976): Studies on the developmental physiology in rice pollen. — Proc. Crop. Sci. Soc. Tokyo. *45*, 279-287.

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PALEOPHYTOGEOGRAPHY OF THE ANGIOSPERM POLLEN GRAINS DURING THE UPPER CRETACEOUS AND THE TERTIARY I

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Abstract

The Upper Cretaceous — Paleogene paleophytogeographical regions are based on the distribution of the pollen grains of *Normapolles, Triprojectacites, Oculata* Group, *Proteaceae, Ulmaceae,* and *Olacaceae*. The pollen group *Postnormapolles* was not sufficiently studied from this point of view. This paper deals with the regional distribution during the geological past of one form-species of early type from the genuses *Triatriopollenites* and *Triporopollenites*, all species of the genus *Platycaryapollenites* and *Paraalnipollenites. Triatriopollenites roboratus* has the most restricted area and occurrence in the geological time units, namely this species occur in the Paleocene — Eocene of Eurasia. *Triporopollenites robustus* is also mostly from Paleocene — Eocene age but there are scattered data from the Upper Cretaceous and from the Eocene until the Miocene. This species occur not only in Eurasia, but in North-America and North-Africa too. The pollen grains of the genus *Paraalnipollenites* were mainly the elements of the province *Aquilapollenites*, and the intermediate region, its occurrence in the *Normapolles* province is scarce. This form-genus seems to be charasteristic for the Upper Cretaceous, Paleocene and Eocene time, but there are data from younger sediments too. The pollen grains of the genus *Platycaryapollenites* appear in the Upper Cretaceous (North-Africa). It is very common in the Paleocene, but its largest distribution is during the Eocene, important in the Oligocene. During the Neogene its area become restricted gradually. From the Pliocene it is only a single occurrence from the Southern Hemisphere from a deep sea drilling near the Timor Islands.The pollen grains analyzed in this paper may be derived from the *Normapolles* group. But its distribution overstep the boundaries of the *Normapolles* taxa, in this way its radiation was very rapid during the Upper Cretaceous and the lower part of the Paleogene.
Key words: Palynology, Paleophytogeography, Cretaceous — Tertiary.

Introduction

The study of the regional distribution of several sporomorph taxa during the geological past may be considered as a method in Palynology. SAKS et al., (1973) emphasized that these investigations are important in the reconstruction of the paleophytogeographical environments, and in the establishment of the paleoclimatic zones and the paleopole. As pioneering publications, dealing with the Upper Cretaceous — Paleogene regions based on palynological data the importance of the paper of KRUTZSCH (1960) and ZAKLINSKAYA (1962) must be emphasized. Concerning the paleophytogeographical units, which were established in these classical publications, later several supplementary data were published by several

authors: MTCHEDLISHVILI and SAMOILOVICH (1962), ZAKLINSKAYA (1963, 1966, 1967a,b, 1976), SHAKHMOUNDES (1966), SAMOILOVICH (1967), KEDVES and KIRÁLY (1968, 1970), STANLEY (1970), KHLONOVA (1971), WIGGINS (1976), SRIVASTAVA (1978), and BATTEN (1981). As comprehensive papers, the publications of MÉDUS (1973), HERNGREEN and KHLONOVA (1981) and KEDVES and DINIZ (1983) may be mentioned. Important data were published on this point of view by ROCHE (1974). A new paleophytogeographical synthesis for the Upper Cretaceous was published by the present writer (KEDVES 1985). The paleophytogeographical provinces and districts for the Paleocene, Eocene and Oligocene were elaborated by BOITSOVA and PANOVA (1973).

During the Tertiary, the extraordinarily differentiated Upper Cretaceous and Paleogene floras were altered, with a more uniform vegetation. The early angiosperms disappeared, these taxa are represented first in Europe by the *Normapolles* group, which has a very complicated germinal exine structure. Its place was occupied by the *Postnormapolles*, by the developed types of the *Longaxones*, and other different kinds of angiosperms.

The pollen group of the *Postnormapolles* may be derived at least in its greater part from the *Normapolles*. But these pollen grains occur not only in the *Normapolles* (European — Turanian; KHLONOVA, 1971) province, but in such territories where *Normapolles* have not existed, or their occurrence is very scarce. In the case of some *Postnormapolles* a heterogeneous origin may be presumed. This

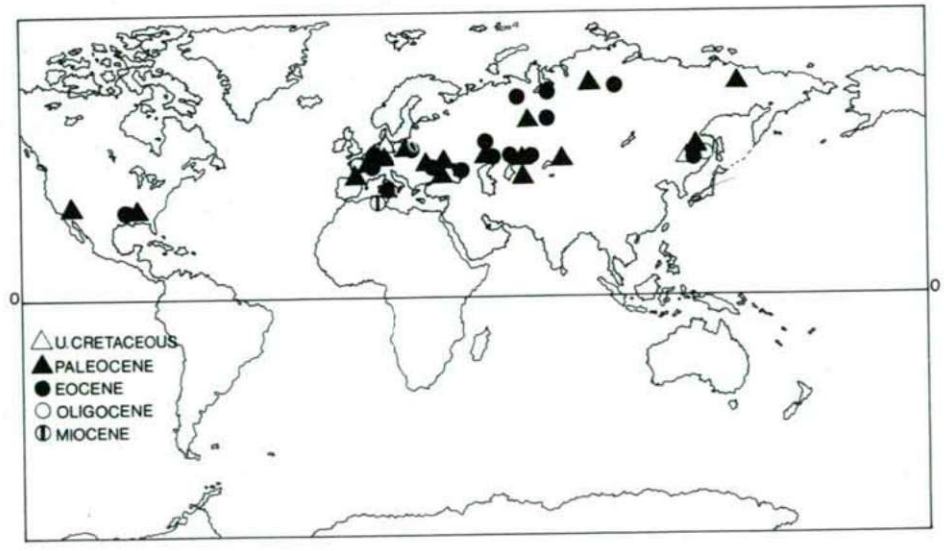


Fig. 1. Regional distribution of *Triporopollenites robustus* PF. 1953 during the Upper Cretaceous and the Tertiary.

problem was focussed during our present research project, by the way of the regional and chronological distribution of several form-species and form-genuses. This paper summarizes the first results concerning this subject.

Methods

The literature data, which were available for us was evaluated and documented for each taxa. During the collection of the data, the following problems arise:

1. In several cases there are doubts in the determinations of the sporomorphs.
2. In some papers the precision of the geological age of the spore-pollen assemblage is not satisfactory.
3. Sometimes it was not easy to establish the localities. On the other hand there were given very large territories, on the other so small geographical objects were given, which are not indicated on the large world maps.

It was impossible to indicate all the collected data, because the proportion size of our map. But the principal goal was, that each of the most important data for the paleophytogeographical evaluation were indicated on the map.

The objects of this paper were the triporate, and triatriate pollen grains as the most primitive types of the *Postnormapolles*.

Results

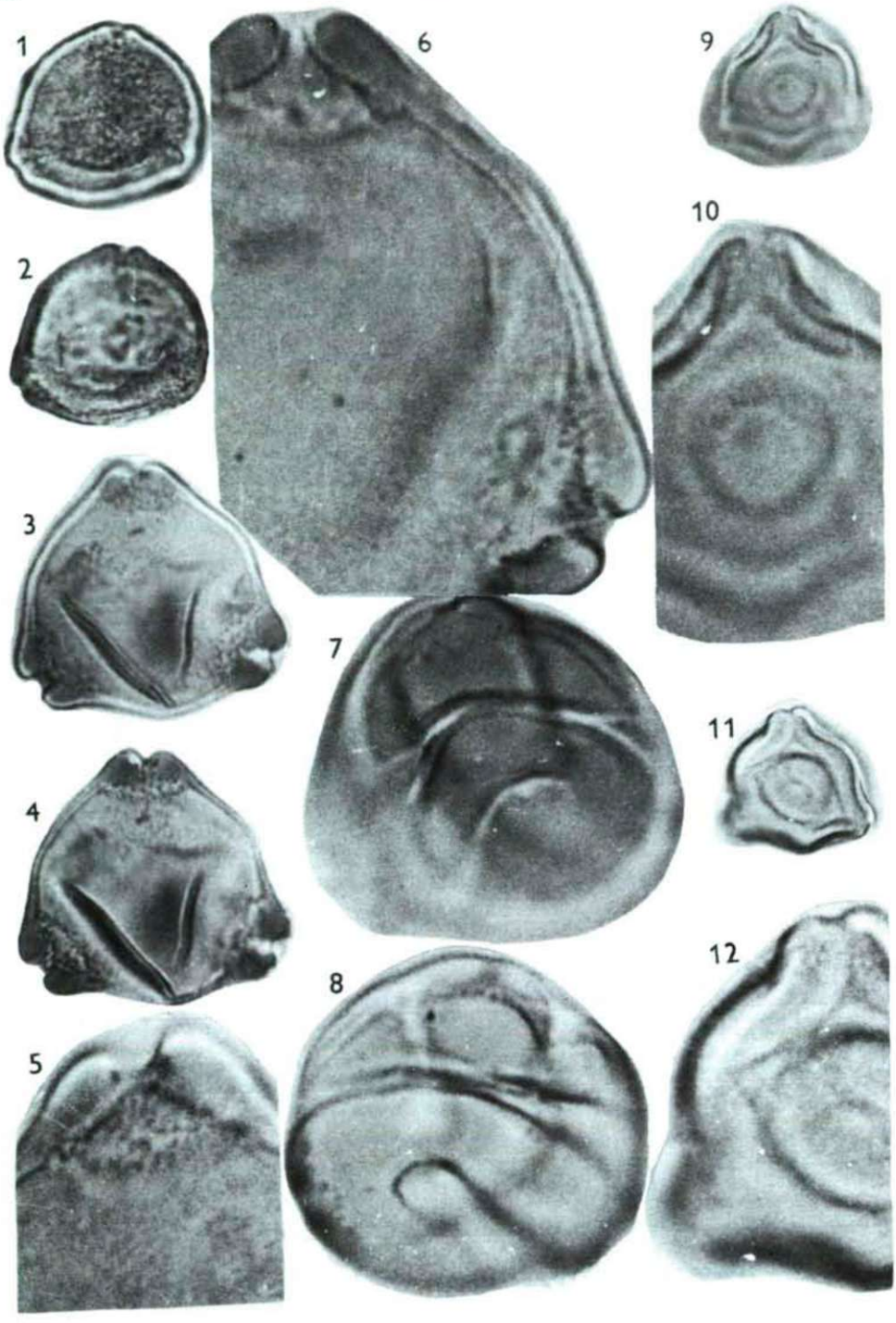
Fgen.: *Triporopollenites* PF. et TH. 1953

Triporopollenites robustus PF. 1953 subfsp. *robustus, Betulaceae,* (Plate I, fig. 1,2)

This kind of pollen grain is a very early triporate type, which probably may be distinguished taxonomically from the other developed pollen grains of this morphological type. TEM data about this species were published by KEDVES and PÁRDUTZ (1970), and by KEDVES and STANLEY (1976), this latter paper deals with the results of the SEM studies too.

The appearance of this form-species is the Upper Cretaceous, we have data from Europe, and from the Far East, in this way this species appeared in the *Normapolles* and *Aquilapollenites* province at the same time (fig. 1). It is worthy to mention that it is not yet known from the intermediate zone between the *Normapolles* and *Aquilapollenites* province in Eurasia, and from North America too. During the Paleocene and Lower Eocene firstly in the lower Eocene in Eurasia the distribution of this species is common. From North America only Paleocene and few Lower Eocene data are known. We have data from the Oligocene of Europe, and from the Far East, but the re-study of these specimens may alter our present-day knowledge concerning the occurrence of this species. The Miocene data from North America is in question.

As regards the origins of this pollen type the *Normapolles* group may be taken first into consideration. But on the basis of its occurrence in the Paleocene it may not be presumed that this species radiated from the European gene centrum to Siberia. Our earlier results (KEDVES and DINIZ, 1979) reveal that between the *Normapolles*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and *Postnormapolles* group there are a number of intermediate types and it may be presumed that it is necessary to create a new group for some early *Brevaxones* pollen grains, which occur not only in the *Normapolles,* but in the *Aquilapollenites* province. On the other hand, the common taxa of the established paleophytogeographical units did not yet come into the limelight, because the subject of the researchers were firstly the segregate angiosperm pollen types.

Fgen.: *Triatriopollenites* PF. 1953

Triatriopollenites roboratus PF. 1953, *Myricaceae* (Plate I, fig. 3—6)

Syn.: 1960 SAUER, in POKROVSKAYA et STELMAK. — *Myrica mirabilis,* sp. nov., p. 412. pl. V, fig. 9a,b.

Its characteristic atrium (Plate I, fig. 5,6) and the exoaperture elongated in polar direction refer to an early *Brevaxones* origin. It is regretful that about this very important pollen grain we have not yet transmission electron microscopical data.

This species is important from stratigraphical point of view of the Paleocene and the Lower Eocene (fig. 2). In contrast to the occurrence in the Paleocene and Lower Eocene layers of the Far East, based on our present day knowledge, this is an element of the *Normapolles* province as an attending of the *Normapolles* taxa. It is noteworthy that against the widespread researches it is not yet found in North America. In this respect, this species has the identic, regional, and pro parte stratigraphic value, with the genus *Stephanoporopollenites,* having a decisive importance in Europe and in the intermediate region in the determination of the Paleocene age, and similarly not yet demonstrated from North America either (KEDVES, 1977). The difference between the stratigraphic value of *Triatriopollenites roboratus,* and the form-genus *Stephanoporopollenites* is, that the taxa of the latter mentioned genus occurred never in the sediments younger as Thanetian in contrast to the Lower Eocene (Sparnatian) occurrence of *Triatriopollenites roboratus.*

Plate I

1,2 *Triporopollenites robustus* PF. 1953 subfsp. *minor* KDS. 1970, cf. *Betulaceae,* slide: Menat-2, cross-table number: 14.7/116.6, following KEDVES and RUSSELL (1982), x1000

3,4 *Triatriopollenites roboratus* PF. 1953, slide: Menat-8, cross-table number: 18.3/120.9, following KEDVES and RUSSELL, (1982) x1000

5,6 *Triatriopollenites roboratus* PF. 1953, slide: Menat-26, cross-table number: 14.6/103.7, following KEDVES and RUSSELL (1982), x2500

7,8 *Platycaryapollenites swasticoidus* (ELSIK 1974) FRED. et CHRIST. 1978, *Juglandaceaea, Platycarya,* slide: Menat-39, cross-table number: 14.2/111.3, following KEDVES and RUSSELL (1982), x2500

9,11 *Paraalnipollenites alterniporus* (SIMPS. 1961) SRIV. 1975, slide: Menat-37, cross-table number: 16.9/117.1, x1000

10,12 *Paraalnipollenites alterniporus* (SIMPS. 1961) SRIV. 1975, slide: Menat-37, cross-table number: 16.9/117.1, x2500

Fgen.: *Platycaryapollenites* E. NAGY 1969 emend. FREDERIKSEN et CHRISTOPHER 1978

Platycaryapollenites swasticoidus (ELSIK 1974) FRED. et CHRIST. 1978, *Juglandaceae, Platycarya* (Plate I, fig. 7,8)

The morphology of these pollen grains is very characteristic. TEM and SEM data about this form-genus were published by KEDVES and STANLEY (1976). The importance of the regional distribution of the pollen grains of this form-genus is supported by the fact that we have publications from this point of view; LEOPOLD and MACGINITIE (1972), GRUAS-CAVAGNETTO (1977). Following ZAKLINSKAYA (1962) the appearance of the genus Platycarya was in the Upper Cretaceous. From Upper Cretaceous layers of Egypt, KEDVES (1971) published as a preliminary report pollen grains belonging to this form-genus. KEDVES and DINIZ (1979) from the layers of Aveiro type spore-pollen assemblage (Santonian-Campanian, Portugal) published cf *Platycaryapollenites semicyclus*, because its endoaperture differ from the pollen grains of the Paleocene and Eocene sediments. In this way the appearance and especially the distribution needs further investigations. It is worthy of mentioning in this place too, that the studies of the sporomorphs of the Upper Cretaceous sediments were interested first in the researches of the *Normapolles* and *Aquilapollenites (Triprojectacites)*, which have a peculiar exine structure.

The regional distribution of the fossil forms of the genus *Platycarya* was published by LEOPOLD and MACGINITIE (1972), this conception was later completed by GRUAS-CAVAGNETTO (1977) with French data. On the basis of the

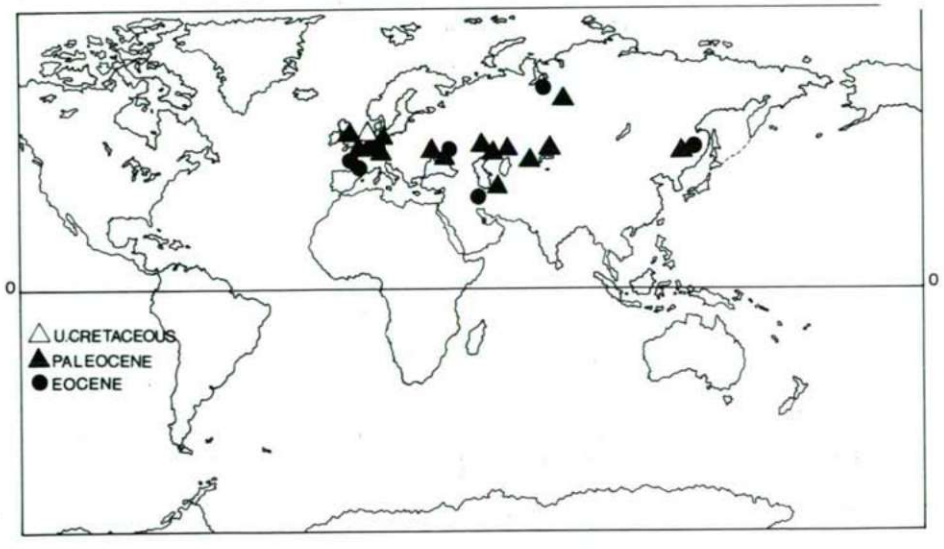

Fig. 2. Regional distribution of *Triatriopollenites roboratus* PF. 1953 during the Upper Cretaceous and the Tertiary.

distribution map of LEOPOLD and MACGINITIE (1972) the acme of this genus is in the Oligocene epoch. Following our new compilation (fig. 3) the pollen grains of this genus was very wide-spread in the Paleocene and the flowering age was in the Eocene. In the Oligocene its importance diminuted, but the pollen grains of this genus were remarkably distributed. As regards the Miocene age our results are identical with those of LEOPOLD and MACGINITIE (1972), namely the reduction of this genus is striking. The disappear in North America, but in Europe occur in several localities. Interesting are data from the Pliocene, in contrast that these are scarce, they came mostly from Europe. Noteworthy is the occurrence, south of the Equator, from a deep-sea drilling, near the Timor Islands.

Fgen.: *Paraalnipollenites* HILLS et WALLACE 1969 here emend

Syn.: 1976, SUNG TZE-CHEN et TSAO LIU. — *Fushunpollis* gen. nov., p. 155.

Fgen. Type: *Paraalnipollenites alterniporus* (SIMPSON 1961) SRIVASTAVA 1975 (Plate I, fig. 9-12)

Syn.: 1961, SIMPSON. — *Alnus alternipora*, p. 443, pl. 13, fig. 5.
1963, ZAKLINSKAYA. — *Triatriopollenites confusus*, p. 232, pl. 34, fig. 7, cf. 6, 8.
1969, HILLS and WALLACE. — *Paraalnipollenites confusus* (ZAKLINSKAYA) n. comb., p. 141, pl. 17, figs. 1-8.
1976, SUNG TZE-CHEN and TSAO LIU. — *Fushunpollis arcuatus* sp. nov., p. 155, 156, pl. II, figs. 32-38.

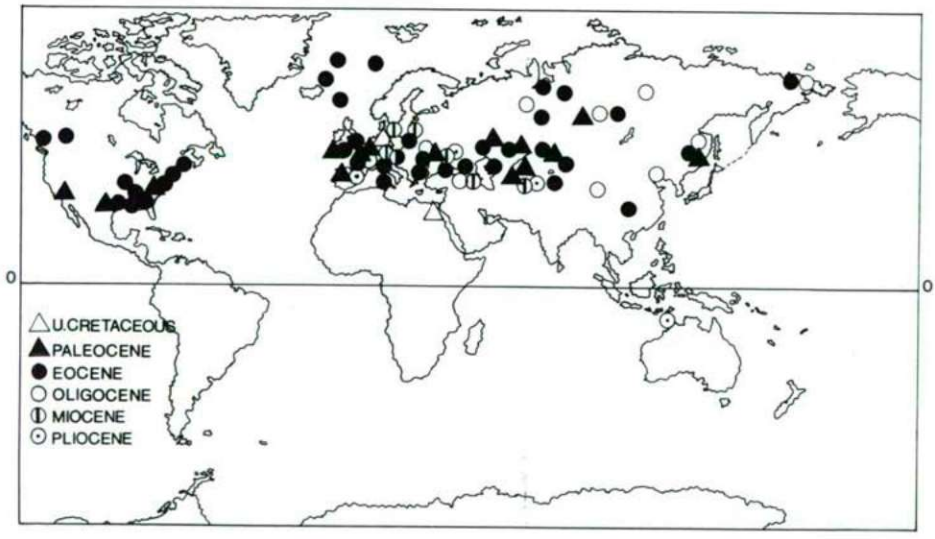

Fig. 3. Regional distribution of *Platycaryapollenites* E. NAGY 1969 emend. FREDERIKSEN et CHRISTOPHER 1978 during the Upper Cretaceous and the Tertiary.

Emended diagnosis

Triatriate pollen grains. On one of the hemispheres there is a thickening form of a ring, and there are arci too. The form and the thickness of the arci is varied. By LM method no characteristic sculpture may be detected.

Remarks. — The pollen grains, which may be classed into this form-genus have four important morphologic characteristic features: 1. the atrium, 2. the polar thickening form a ring, 3. the arci, 4. the more or less smooth surface.

Differential diagnosis

The joint presence of the above enumerated characteristic features well distinguish from the pollen grains of *Triatriopollenites* PF. 1953 and *Plicapollis* PF. 1953.

On the basis of the first data, which were reviewed by HILLS and WALLACE (1969) it seemed that the pollen grains of this form-genus are the elements of the *Aquilapollenites* province, and because of its restricted, Maestrichtian, and Paleocene occurrence, have a stratigraphic importance. The earliest data were published from the Turonian of Europe, this is unique, but no microphotographs accompanied this finding (PACLTOVÁ, 1973) so it was no taken into consideration of the time being. In this way the appearance remain the upper part of the Upper Cretaceous. The youngest occurrences are as follows: Eocene/Oligocene: Far East, Eocene ?Oligocene: China, Region of Bohai. There are well established occurrences from Europe and Siberia. It is interesting that from the Eocene period these pollen grains were not found from localities studied palynologically in North America (fig. 4).

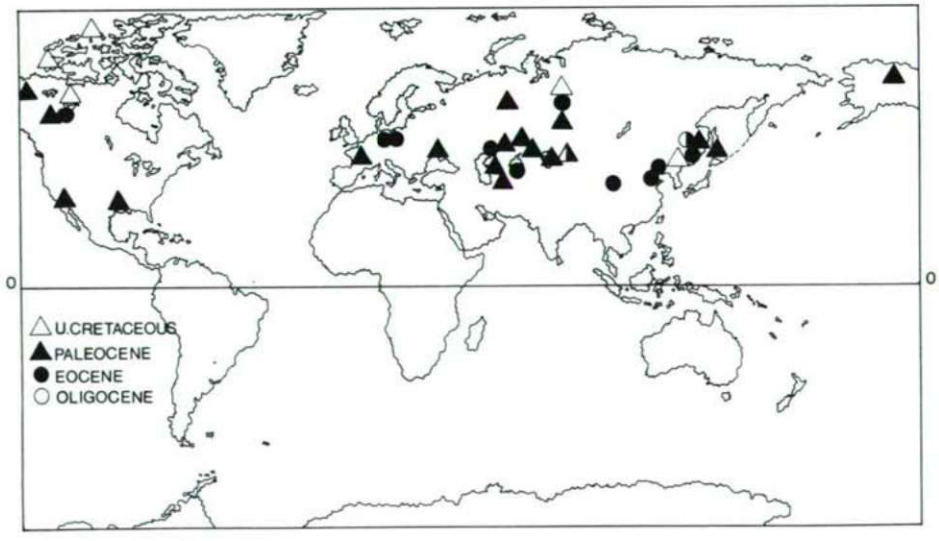

Fig. 4. Regional distribution of *Paraalnipollenites* HILLS et WALLACE 1969 emend. during the Upper Cretaceous and the Tertiary.

The plants producting of these pollen grains have probably very peculiar ecologic pretensions. In Western Europe there is only one occurrence, published from the Paleocene of Menat, France in contrast that there are studied several localities, which are rich in sporomorphs. Similarly, Eocene is also well known in Europe, but this genus was demonstrated only from two localities of Poland, and from the Black Sea Region. In this way the occurrence in Europe in the *Normapolles* province is scarce. Not yet found in the sub-province of the Atlantic Coast of North America.

Conclusions

1. The regional distribution of all *Postnormapolles* taxa discussed in this paper overstep the borders of the *Normapolles* province.
2. The geographical and stratigraphical occurrence of the most ancient types of triporate and triatriate pollen grains reveals to the heterogeneous origin of the *Postnormapolles*.
3. The paleophytogeographic elaboration of the *Postnormapolles*, *Longaxones* and other kind of pollen grains may give new ideas to our knowledge about the vegetation history.

ACKNOWLEDGEMENTS

On my request, SUNG TZE-CHEN (Nanking Instituite of Geology and Palaeontology, Academia Sinica) was kind enough to translate the Chinese text into English about the genus *Fushunpollis*. I express my sincerest thanks for this contribution.

Appendix

The data of the following publications were used for the distribution maps.

AUFFRET — GRUAS-CAVAGNETTO, 1975, Bull. Soc. France *17*, 641-655.; AKHMETIEV, BRATSEVA — VAKHRAMEEV, 1976, Geol. Paleont. Far East, 46-50.; BALKWILL — HOPKINS, 1976, Geol. Surv. Can. Paper 76-1B, 329-334.; BAIBULATOVA, 1968, Paleont. Meth. for Strat., 226-228.; BAIBULATOVA, 1971, Palynology of Kazakhstan, 112-113.; BARBASHINOVA, 1973, The Palynology of Cenophytic, 101-104.; BLYAKHOVA, 1966, For the II. Int. Palynol. Conf., 127-131.; BLYAKHOVA, 1971, Palynology of Kazakhstan, 114-125.; BLYAKHOVA, 1976, Palynology of Kazakhstan, 69-77.; BLYAKHOVA — KALMENEVA — PONOMARENKO, 1971, Palynology of Kazakhstan, 93-106.; BLYAKHOVA — KOVRIZHNYKH, 1971, Dokl. Akad. Nauk SSSR, *200*, 1401-1402.; BOITSOVA — PANOVA, 1967a, Akad. Nauk SSSR, 262-270.; BOITSOVA — PANOVA, 1973b, The Palynology of Cenophytic, 42-47; BOITSOVA — PANOVA, 1976, Trudy VSEGEI 195, 144-179; BOITSOVA, OSHURKOVA — PANOVA, 1973, Methodical Problems of Palynology, 8-12.; BOLOTNIKOVA, 1964, Geology and Geophysics, 140-142.; BOLOTNIKOVA, 1966, For the II. Int. Palynol. Conf., 131-136.; BONDARENKO, 1973, The Palynology of Cenophytic, 8-12.; BRATSEVA, 1965, Akad. Nauk SSSR, 129, 1-42.; BRATSEVA, 1966, For the II. Int. Palynol. Conf., 136-141.; BRATSEVA,

1967, Rev. Palaeobotan. Palynol. 2, 119-126.; BRATSEVA, 1969, Akad. Nauk SSSR, 207, 1-56.; BRELIE, QUITZOW — STADLER, 1969, Fortschr. Geol. Rheinld. u. Westf. *17*, 27-40.; CARATINI 1975, B.S.G.F. *17*, 797-802.; CAVAGNETTO, 1964; Rev. de Micropaléont. *7*, 57-64.; CHATEAUNEUF, 1971, Bull. du B.R.G.M. *1*, 16-19.; CHRISTOPHER, PROWELL, REINHARDT — MARKEWICH, 1980, Palynology *4*, 104-124.; DEMARCQ, MÉON-VILAIN, MIGUET — KUJAWSKI, 1976, Serv. Géol. de Tunisie, *42*, 97-147.; DISTANOV — KUSNETZOVA, 1971, Trud. Geol. Inst. (Kazan) *20*, 234-239.; DOERENKAMP, JARDINÉ — MOREAU, 1976, Bulletin of Canadian Petroleum Geology *24*, 372-417.; DRUGG, 1967, Palaeontographica *B*, *120*, 1-71.; DURAND, 1957, C.R. des séances de l'Acad. Sci. *245*, 2067-2069.; DURAND, 1958, C.R. des séances de l'Acad. Sci. *247*, 1753-1776.; DURAND, 1960a, Soc. Geol. et Min. de Bretagne N.S. *2*, 71-80.; DURAND, 1960b, Mém. de la Soc. Géol. et Miner. de Bretagne 389.; DURAND, 1962, Colloque sur le Paleogène, 1001-1008.; DZHABAROVA, 1973, The Palynology of Cenophytic, 173-177.; ELSIK 1974, Palaeontographica *B*, *149*, 90-111.; ELSIK — DILCHER, 1974, Palaeontographica *B*, *146*, 65-87.; ENGELHARDT, 1964, Mississippi Geol. Res. Papers *104*, 65-95; ESENALINOV — PASKAR, 1976, Palynology of Kazakhstan, 78-81.; FAIRCHILD — ELSIK, 1969, Palaeontographica *B*, *128*, 81-89.; FRADKINA — BARANOVA, 1973, The Palynology of Cenophytic, 152-156.; FRADKINA, KISELEVA, ERMOLAEVA, ZHABREVA — ZHARKOVA, 1971, Cenozoic flore of Siberia, on palynological basis, 22-39.; FREDERIKSEN, 1977, US Dept. of the Int. Geol. Surv. 1-26.; FREDERIKSEN, 1979, Palynology, *3*, 129-169.; FREDERIKSEN, 1980, Geol. Surv. Prof. Paper, 1084.; FREDERIKSEN, BYBELL, CHRISTOPHER, CRONE, EDWARDS, GIBSON, HAZEL, REPETSKI, RUSS, SMITHPAUD — WARD, 1982, Tulane Stud. Geol. and Paleont. *17*, 23-45.; FREDERIKSEN, 1983, Cenoz. Geol. 23-31.; FREDERIKSEN, 1984a, Cretaceous and Tertiary etc., 163-168.; FREDERIKSEN, 1984b, Geol. Surv. Prof. Paper, 1308.; GAPONOFF, 1984, Palynology, *8*, 71-106.; GÓCZÁN, 1964, Acta Geol. *8*, 229-264.; GRABOWSKA, 1965, Kwartalnik Geologiczny *9*, 815-836.; GRABOWSKA, 1968, Kwartalnik Geologiczny, *12*, 155-166.; GRABOWSKA, 1973, Instytut Geologiczny *281*, 67-90.; GRABOWSKA — PIWOCKI, 1975, Instytut Geologiczny *284*, 41-70.; GRUAS-CAVAGNETTO, 1966, Rev. de Micropaléont. *9*, 57-67.; GRUAS-CAVAGNETTO, 1967, Rev. Palaeobotan. Palynol. *5*, 243-261.; GRUAS-CAVAGNETTO, 1968a, Mém. du B.R.G.M. *59*, 113-159.; GRUAS-CAVAGNETTO, 1968b, Mém. de la Soc. Géol. de France *47*, 1-144.; GRUAS-CAVAGNETTO, 1969, C.R. Sommaire des Séances de la Soc. Géol. de France *6*, 221-222.; GRUAS-CAVAGNETTO, 1970a, Rev. de Micropaléont. *13*, 69-78.; GRUAS-CAVAGNETTO, 1970b, Pollen et Spores *12*, 71-82.; GRUAS-CAVAGNETTO, 1970c, C.R. Sommaire des Séances de la Soc. Géol. de France *1*, 19-20.; GRUAS-CAVAGNETTO, 1972, Rev. de Micropaléont. *15*, 63-74.; GRUAS-CAVAGNETTO, 1973, Paléobiologie continentale *4*, 1-13.; GRUAS-CAVAGNETTO, 1974, B.S.G.F. *16*, 89-90.; GRUAS-CAVAGNETTO, 1976, Cahiers de Micropaléontologie *1*, 1-49.; GRUAS-CAVAGNETTO, 1978, Mém. de la Soc. Géol. de France N.S. *56*, 131, 1-64.; HILLS — WALLACE, 1969, Geol. Survey of Canada, 182, 139-145.; KEDVES, 1967, Acta Biol. Szeged. *13*, 11-23.; KEDVES, 1968, Pollen et Spores *10*, 117-128.; KEDVES, 1970a, Acta Biol. Szeged. *16*, 51-54.; KEDVES, 1970b, Pollen et Spores *12*, 83-97.; KEDVES, 1971, Acta Bot. Acad. Sci. Hung. *17*, 371-376.; KEDVES, 1974, Studia Biol. Acad. Sci. Hung. *13*, 1-124.; KEDVES, 1978, Acta Biol. Szeged. *24*, 23-30.; KEDVES under publication, KEDVES — STANLEY, 1976, Pollen et Spores *18*, 105-127.; KEDVES — HERNGREEN, 1980, Pollen et Spores *22*, 483-544.; KEDVES — RUSSELL, 1982, Palaeontographica *B*, *182*, 87-150.; KHAIKINA — BELAIA, 1968, Paleopalynological Method for Stratigraphy, 157-163.; KONZALOVÁ — STUHLIK, 1983, Cas. pro miner. geol. *28*, 363-378.; KORALLOVA, 1962, Ukr. Bot. J. *19*, 55-62.; KORALLOVA, 1964, Izv. vys. etc. *5*, 138-140.; KORALLOVA, 1966a, Mat. for the II. Int. Palynol. Conf., 6. 82-85.; KORALLOVA, 1966b, For the II. Int. Palynol. Conf., 141-148.; KORALLOVA, 1968, Thesis, Leningrad, 1-16.; KORALLOVA, 1971, Problems of Palynology *1*, 129-138.; KORALLOVA, 1973, The Palynology of the Cenophytic, 52-55.; KORENEVA, ZAKLINSKAYA, BRATZEVA — KARTASHOVA, 1976, Initial Reports of the Deep Sea Drilling Project *38*, 1169-1193.; KRUTZSCH, PCHALEK — SPIEGLER, 1960, Internat. Geol. Congr. XXI. sess., 135-143.; KRUTZSCH — VANHOORNE, 1977, Palaeontographica, *B*, *163*, 1-110.; KUNERT — LENK, 1964, Geologie *13*, 403-428.; KULKOVA — LAUKHIN, 1975, Acad. Sci. URSS Siberian Sect. *225*, 1-86.; KUSNETZOVA, 1965a, Pollen et Spores *7*, 533-538.; KUSNETZOVA, 1965b, Bull. Soc. Naturalists (Moscow) Geol. Div. *40*, 75-79.; KUSNETZOVA, 1966, Dokl. Acad. Sci. URSS *168*, 1135-1137.; KUSNETZOVA, 1968, Paleopalynological Method for Stratigraphy, 166-174.; KUSNETZOVA, 1970, Dokl. Acad. Sci. URSS *190*, 169-172.; KUSNETZOVA, 1973, The Palynology of the Cenophytic, 55-60.; KUSNETZOVA, 1974, Dokl. Acad. Sci. URSS *2*, 417-419.; LEYE, 1968, Paleopalynological Method for Stratigraphy, 175-187.; LEYE, 1973, The

Palynology of the Cenophytic, 60-65.; MACKO, 1957, Prace Wroclawskiego Towarzistwa Naukowego *B, 88*, 1-313.; MACKO, 1961, The New Phytologist *60*, 207-210; MANIKIN, 1966, For the II. Int. Palynol. Conf. 173-178.; MAZANCOVÁ, 1960, Cas. pro miner. a geol. *5*, 265-275.; MCINTYRE, 1974, Geol. Survey of Canada, *74-14*, 1-56.; MÉDUS, 1977, Géobios, *10*, 625-639.; MÉDUS, 1978, Palinologia, *1*, 341-353.; NAGY, 1969, Ann. Inst. Geol. Publ. Hung. 237-537.; NAGY, 1973, The Palynology of the Cenophytic, 162-165.; NAGY — RÁKOSI, 1966, M.Á.F.I. évi jelentése az 1964 évről, 265-283.; NESTEROVA, 1971, Palynology of Kazakhstan, 107-111.; ODIN, BLONDEAU, DAMOTTE, DURAND, OLLIVIER-PIERRE, LE CALVEZ, LEZAUD, PERREAU — POMEROL, 1972, Bull. d'Inf. des Geol. du Bassin de Paris *32*, 21-52.; OLLIVIER-PIERRE, 1970, Thèse, Fac. Sci. Univ. de Rennes, 1-175.; OLLIVIER-PIERRE, 1980, Mém. Soc. géol. miner. Bretagne *25*, 1-239.; PACLTOVÁ — SIMONCSICS, 1970, Palaeont. Abh. *B, 3*, 599-617.; PANOVA, 1967, Biostrat. Sborn. *115*, 41-59.; PANOVA, 1968, Paleopalynological Method for Stratigraphy, 206-219.; PANOVA, 1971, Cenozoic flore of Siberia on palynological basis, 40-50.; PAKHAMOV, 1976, Palynology of URSS, 112-116.; PITTAU, 1977, Bol. della Soc. Paleont. Italiana *16*, 3-14.; POGODAEVA — ORLOV, 1968, Paleopalynological Method for Stratigraphy, 175-187.; POKROVSKAYA — STELMAK, 1960; Leningrad; POLUMISKOVA, TEREKHOVA, BLYAKHOVA — PONOMARENKO, 1966, For the II. Int. Palynol. Conf.; 154-158.; PONOMARENKO, 1966, For the II. Int. Palynol. Conf., 154-158.; PORTNIAGINA, 1966, For the II. Int. Palynol. Conf., 158-164.; POTONIÉ, 1931, Jb. der Preuss. Geol. Landesanst. f. 1931, *52*, 1-4.; RAMISHVILI, 1969, Tbilisi, 1-131.; RAMISHVILI, 1976, Palynology of URSS, 119-122.; RÁKOSI, 1966, M.Á.F.I. évi jelentése az 1964 évről, 377-387.; RÁKOSI, 1973, Ann. Inst. Geol. Publ. Hung. *55*, 497-697.; RÁKOSI, 1979, M.Á.F.I. évi jelentése az 1977 évről, 241-256.; ROCHE, 1965, Bull. Soc. belge Géol. Paléont. d'Hydrol. *73*, 423-444.; ROCHE, 1966, Bull. Soc. belge Géol. Paléont. d'Hydrol. *74*, 411-420.; ROCHE, 1969, Bull. Soc. belge Géol. Paléont. d'Hydrol. *78*, 131-146.; ROCHE, 1972, Bull. Soc. belge Géol. Paléont. d'Hydrol. *81*, 183-189.; ROCHE, 1973a, Bull. Acad. royale de Belgique, 956-969.; ROCHE, 1973b, Bull. Soc. belge Géol. Paléont. d'Hydrol. *82*, 487-495.; ROCHE, 1973c, Mém. Expl. Cartes Géol. de la Belgiques *13*, 1-138.; ROCHE — SCHULER, 1976, Service Geol. de Belgique Prof. Paper 1976/11, 1-57.; ROTMAN, 1971, Problems of Palynology *1*, 117-128.; ROTMAN, 1973, The Palynology of the Cenophytic, 47-52.; ROUSE, HOPKINS, jr. — PIEL, 1971, Geol. Soc. of America, Special Paper *127*, 213-246.; ROUSE — SRIVASTAVA, 1972, Canad. J. of Earth Sci. *9*, 1163-1173.; RYBAKOVA, 1966, For the II. Int. Palynol. Conf., 178-184.; SCHUMACKER-LAMBRY — ROCHE, 1973, Ann. Soc. Géol. de Belgique *96*, 413-433.; SCHUMACKER-LAMBRY — VANGUESTAINE, 1977; SHAKHMUNDES, 1966, Mat. for the II. Int. Palynol. Conf., 172-195.; SHEKINA, 1953, Bot. J. Ukr.SR *10*, 44-61.; SHEKINA, 1954, Bot. J. *11*, 89-108.; SHEKINA, 1964, Ukr. Bot. J. *21*, 61-69.; SHEKINA, 1969, Bot. J. *26*, 39-47.; STAPLIN, 1976, Bull. Canad. Petroleum Geol. *24*, 117-130.; STREEL, BICK, FAIRON-DEMARET, SCHUMACKER-LAMBRY — VANGUESTAINE, 1977; SUC, 1976, Revue de Micropaléontologie *18*, 246-255.; SUC, 1980, Thèse Univ. Sci. et Techn. du Languedoc; SONG ZHI-CHEN — CAO LIU, 1980, Paper for the 5th Int. Palynol. Conf., 1-10.; SUN XIUYU, ZHAO YINGNIANG — HE ZHUOSHENG, 1984, Geol. Rev. *30*, 207-216.; SUNG TZE-CHEN — TSAO LIU, 1976, Acta Paleontologica Sinica *15*, 147-162.; SUNG TZE-CHEN — TSAO LIU, 1978, Nanjing Inst. Geol. Paleont. Acad. Sinica, 1-177.; TAKAHASHI — JUX, 1982, Bull. Fac. Liberal Arts, Nagasaki Univ. *23*, 23-134.; THIELE-PFEIFFER, 1979, Ph.D. München; THOMSON — PFLUG, 1953, Palaeontographica *B, 94*, 1-138.; TSCHUDY, 1973, Geol. Surv. Prof. Paper 743-B, 1-24.; TSCHUDY — PATTERSON, 1975, Jour. Res. U.S. Geol. Surv. *3*, 437-445.; VIENOZINSKIENE, 1960, Int. Geol. Cong. XXI sect., 247-252.; ZAKLINSKAYA, 1962, For the First Int. Palynol. Conf., 127-131.; ZAKLINSKAYA, 1963, Acad. Sci. URSS, Trud. Geol. Inst. *74*, 1-156.; ZAKLINSKAYA, 1967, Abh. zentr. geol. Inst. *10*, 183-187.; ZAKLINSKAYA, 1975, Izv. Acad. Sci. URSS, Ser. Geol. *6*, 110-121.; ZAKLINSKAYA, 1976, Geol. Paleont. Far East, 51-65.; ZAKLINSKAYA, 1978, Rev. Palaeobot. Palynol. *26*, 227-247.; ZAKLINSKAYA — BRATZEVA, 1973, The Palynology of the Cenophytic, 69-72.; ZAKLINSKAYA — LEYE, 1968, Dokl. Acad. Sci. URSS, *180*, 181-185.; ZHAO YINGNIANG, SUN XIUYU — WANG DANING, 1982, Bull. Inst. Geol. Chinese Acad. Sci. *4*, 95-125.; ZHEZHEL, 1973, Trud VSEGEI *195*, 180-184.; ZIEMBINSKA-TWORZYDLO, 1974, Acta Paleontologica Polonica *19*, 309-432.; ZIVA, 1973, The Palynology of Cenophytic, 89-93.; YU JINGXIAN, GUO ZHENGYING — MAO SHAOZHI, 1983.

References

BARBASHINOVA, V.N. (1973): Pollen of Angiospermae in Paleocene deposits of the Far East, and some new species of formal genus *Triatriopollenites* PFLUG. — The Palynology of the Cenophytic, 101-104. (Russian).

BATTEN, D.J. (1981): Stratigraphic, palaeogeographic and evolutionary significance of Late Cretaceous and early Tertiary *Normapolles* pollen. — Rev. Palaeobot. Palynol. *35*, 125-137.

BOITSOVA, E.P. and PANOVA, L.A. (1973): Paleogene floras and vegetation in the Euro-Asian botanical-geographical province. — The Palynology of the Cenophytic, 42-47. (Russian).

FREDERIKSEN, N.O. and CHRISTOPHER, R.A. (1978): Taxonomy and biostratigraphy of late Cretaceous and Paleogene triatriate pollen from South Carolina. — Palynology *2*, 113-145.

GRUAS-CAVAGNETTO, C. (1977): Étude palynologique de l'Éocène du Bassin Anglo-Parisien. — Thèse de Doct. d'État ès Sci. Nat. Paris, 1-287.

HERNGREEN, G.F.W. and CHLONOVA, A.F. (1981): Cretaceous microfloral provinces. — Pollen et Spores *23*, 441-555.

HILLS, L.V. and WALLACE, S. (1969): *Paraalnipollenites*, a new form genus from Uppermost Cretaceous and Paleocene rocks of Arctic Canada and Russia. — Geol. Survey of Canada *182*, 139-145.

KEDVES, M. (1970): Études palynologiques des couches du Tertiaire inférieur de la Région Parisienne. V. Pollens triporés subtriporés et intratriporés. — Pollen et Spores *12*, 83-97.

KEDVES, M. (1971): Présence de types sporomorphes importants dans les sédiments préquaternaires Egyptiens. — Acta Bot. Acad. Sci. Hung. *17*, 371-376.

KEDVES, M. (1977): Electronmicroscopical examinations of fossil *Angiospermatophyta* pollen grains from the Paleocene and the middle Eocene. — Acta Bot. Acad. Sci. Hung. *23*, 97-103.

KEDVES, M. (1985): The present day state of Upper Cretaceous palaeophytogeography on palynological evidence. — Acta Biol. Szeged. *31*, 115-127.

KEDVES, M. (en cours de publication): Études palynologiques des couches du Tertiaire inférieur de la Région Parisienne. VII.

KEDVES, M. et DINIZ, F. (1983): Contribution à la connaissance des pollens d'Angiospermes du Crétacé supérieur du Portugal. — Bol. Soc. Geol. de Portugal *22*, 19-31.

KEDVES, M. et KIRÁLY, E. (1968): A propos de régions paléophytogéographiques du Crétacé et du Paléogène, d'après les données palynologiques. — Acta Biol. Szeged. *14*, 19-28.

KEDVES, M. et KIRÁLY, E. (1970): Problems of Cretaceous-Palaeogene palaeophytogeographical regions based on palynological results II. — Acta Biol. Szeged. *16*, 63-72.

KEDVES, M. et PÁRDUTZ, Á. (1970): Études palynologiques des couches du Tertiaire inférieur de la Région Parisienne VI. Ultrastructure de quelques pollens d'Angiospermes de l'Éocène inférieur. (Sparnacien). — Pollen et Spores *12*, 553-575.

KEDVES, M. and STANLEY, E.A. (1976): Electronmicroscopical investigations of the *Normapolles* group and some other selected European and North American Angiosperm pollen II. — Pollen et Spores *18*, 105-127.

KHLONOVA, A.F. (1971): Interpretation of paleofloristic differentiations in the spore and pollen complex of North Asia in the Upper Cretaceous period. — Inst. Geol. and Geophys., Acad. Sci. Siberia *8*, 19-28. (Russian).

KRUTZSCH, W. (1960): Über *Thomsonipollis magnificus* (TH et PF. 1953) n. fgen. n. comb. und Bemerkungen zur regionalen Verbreitung einiger Pollen-gruppen im älteren Paläogen. — Freiberger Forschungshefte *86*, 54-65.

LEOPOLD, E.B. and MACGINITIE, H.D. (1972): Development and affinities of Tertiary floras in the Rocky Mountains. Floristics and Paleofloristics of Asia and Eastern North America. — Elsevier Publishing Co., Amsterdam, 147-200.

MÉDUS, J.: Contributions à la géobotanique de l'Eurasie au Crétacé supérieur. — C.R. du quatre-vingt-seizième Congrès National des Sociétés Savantes *5*, 223-232.

MILDENHALL, D.C. and HARRIS, W.F. (1971): Status of *Haloragacidites* (al. *Triorites) harrisii* COUPER) HARRIS comb. nov. and *Haloragacidates trioratus* COUPER 1953. — New Zealand Journal of Botany *9*, 297-306.

MTCHEDLISHVILI, N.D. and SAMOILOVICH, S.R. (1962): Common elements in Mesozoic and Cenozoic floras of Western Siberia and Australia. — Internat. Conf. on Palynology, Tucson (Arizona) Abstr. — Pollen et Spores *4*, 365-366.

NAGY, E. (1969): Palynological investigations of the Miocene in the Mecsek Mountains. — M.Á.F.I. Évkönyve *52*, 235-649.

PFLUG, H.D. (1953): Zur Entstehung und Entwicklung des angiospermiden Pollens in der Erdgeschichte. — Palaeontographica *B*, *95*, 60-171.

PACLTOVÁ, B. (1973): Evolution of Angiosperm pollen of the Bohemian Upper Cretaceous and its time-correlation significance. — The Palynology of the Cenophytic, 24-27.

POKROVSKAYA, I.M. and STELMAK, N.K. (1960): Atlas of Upper Cretaceous, Paleocene and Eocene spore-pollen complexes from various regions of the USSR. — Trud. VSEGEI *30*, 1-575. (Russian).

ROCHE, E. (1974): Paléobotanique, Paléoclimatologie et dérive des continents. — Sci. Géol. Bull. *27*, 9-24.

SAKS, V.N., ILINA, V.K., KULKOVA, I.A. and KHLONOVA, A.F. (1973): Palynology and Paleogeography. — Problems of Palynology, 35-43. (Russian).

SAMOILOVICH, S.R. (1967): Tentative botanico-geographical subdivision of northern Asia in Late Cretaceous time. - Rev. Palaeobotan. Palynol. *2*, 127-139.

SHAKHMUNDES, V.A. (1966): Floristic relations between the northern part of Western Siberia and Europe during the early Eocene. — Methods of paleopalynological investigations, 172-195. (Russian).

SRIVASTAVA, S.K. (1978): Cretaceous spore-pollen Floras: A global evaluation. — Biological Memoirs *3*, 1-130.

STANLEY, E.A. (1970): The stratigraphical, biogeographical, paleont-ecological and evolutionary significance of the fossil pollen group *Triprojectacites*. — Bull. of the Georgia Academy of Science *28*, 1-44.

SUNG TZE-CHEN and TSAO LIU (1976): The Paleocene spores and pollen grains from the Fushun coalfield, Northeast China. — Acta Palaeontologica Sinica *15*, 147-162. (Chinese, with English summary).

THOMSON, P.W. und PFLUG, H.D. (1953): Pollen und Sporen des mitteleuropäischen Tertiärs. — Palaeontographica *B*, *94*, 1-138.

WIGGINS, V.D. (1976): Fossil oculata pollen from Alaska. — Geoscience and Man *15*, 51-76.

ZAKLINSKAYA, E.D. (1962): The value of angiospermous pollen for the stratigraphy of the Upper Cretaceous and Paleogene and botanical-geographical provinces on the Cretaceous-Paleogene boundary. — For the First Int. Palynol. Conf., 105-112.

ZAKLINSKAYA, E.D. (1963): Angiospermous pollen grains and their value for Upper Cretaceous and Paleogene stratigraphy. — Acad. Sci. URSS, Trud. Geol. Inst. *74*, 1-226. (Russian).

ZAKLINSKAYA, E.D. (1966): Pollen morphology of Angiosperms and paleofloristic areas and provinces at the boundary of the Cretaceous and Paleogene. — The Paleobotanist *15*, 110-116.

ZAKLINSKAYA, E.D. (1967a): Palynological studies on Late Cretaceous — Paleogene floral history and stratigraphy. — Rev. Palaeobotan. Palynol. *2*, 141-146.

ZAKLINSKAYA, E.D. (1967b): The Early-Paleogene flora of the Northern Hemisphere and paleofloristic provinces of this age. — Abh. zentr. geol. Inst. *10*, 183-187.

ZAKLINSKAYA, E.D. (1976): Relationship between difference and similarity of Cenophyte flora major components from the point of view of continents movement theory. — Palynology in USSR, 83-87. (Russian).

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IN VITRO DESTRUCTION OF THE EXINE OF RECENT PALYNOMORPHS II

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Abstract

We carried out the greater part of the experiments used first for the pollens of *Corylus avellana* on the pollen grains of *Taxus baccata*. The present results differ from those got on the pollen grains of *Corylus avellana*, namely well defined globular biopolymer units were not detectable on the experimentally degraded exine of *Taxus baccata*. In this way the present results support the statement of other authors obtained by chemical methods, there are differences in the chemical structures of the exines of the gymnosperms and angiosperms.
Key words: Taxus baccata, exine, experimental destruction.

Introduction

The first part of this series of publication (KEDVES, 1986) dealt with the most important results of the chemical composition of the sporopollenin reviewed. The present day state, and the problems of the researches of the molecular structure of the sporopollenin were also discussed. It is necessary to emphasize, in this place too, that the results got by different kinds of experiments gave different results. This is, in all probability a consequence of the complicated character of this field of investigations.

We cite the most important results of the earlier experiments on the pollen grains of *Corylus avellana* L., KEDVES (1986): "1. *Helix* enzyme with merkaptoethanol is suitable to decompose the sporopollenin of recent and fossil plant microfossils. 2. The partially decomposed wall, studied by the TEM method, may reveal the molecular structure of the sporopollenin. 3. Our results on recent *Corylus avellana* L. pollen grains suggest a globular structure of the biopolymers of the sporopollenin of this species." This paper, as the second part of this series of publications summarize the results achieved on the exines of the pollen grains of *Taxus baccata* L..

Material and Methods

Among the *Gymnospermatophyta* pollen grains an inaperturate type seemed to be the most suitable for the first experimental object. *Taxus baccata* L. from the *Taxopsida* was chosen, because this species have several peculiar characters, e.g.: the structure of the staminate inflorescence, lack of the cone, the characteristic arillus of the seed, and its alcaloids. In this way it may be presumed that there are differences in the chemical composition of the sporopollenin of the pollen grains of *Taxus baccata*, which will be appear in the molecular structure, too, in contrast to those demonstrated earlier.

Fresh pollen grains of *Taxus baccata* L. were collected 20 March, 1984 on Honvéd square, Szeged, by I. DÁVID. Only mature pollen grains, fallen out from the stamina were the subject of our experiments. The pollen material was placed into dark glass containers. The series of experiments are identical with series no 2. and 3. achieved earlier on the pollen grains of *Corylus avellana*. The so-called experimental series no 2, were complete on 15th May 1984, as follows:

T-5 — 20 mg. air dried pollen grains + 20 ml H_2O dest., temperature 30 °C, length of time: $2^h30'$.

T-5A — the same, only the length of time was 5^h.

T-7 — 20 mg. air dried pollen grains + 2 ml *Helix* enzyme 2%, temperature 30 °C, length of time: $2^h30'$.

T-7A — the same, only the length of time was 5^h.

T-9 — 20 mg. air dried pollen grains + 2 ml *Helix* enzyme 2%, + 20 µl merkapto-ethanol, temperature 30 °C, length of time: $2^h30'$.

T-9A — the same, only the length of time was 5^h.

T-11 — 20 mg. air dried pollen grains + 2 ml *Helix* enzyne 2% + 20 µl merkapto-ethanol + 20 mg. EDTA, temperature 30 °C, length of time: $2^h30'$.

T-11A — the same, the length of time was 5^h.

The second series of experiments (in the case of *Corylus avellana* the 3rd) were done on 26th September, 1984, as follows:

T-4b1 — 20 mg. air dried pollen grains + 1 ml merkapto-ethanol + 2 ml H_2O dest., temperature 30 °C, length of time: $2^h30'$.

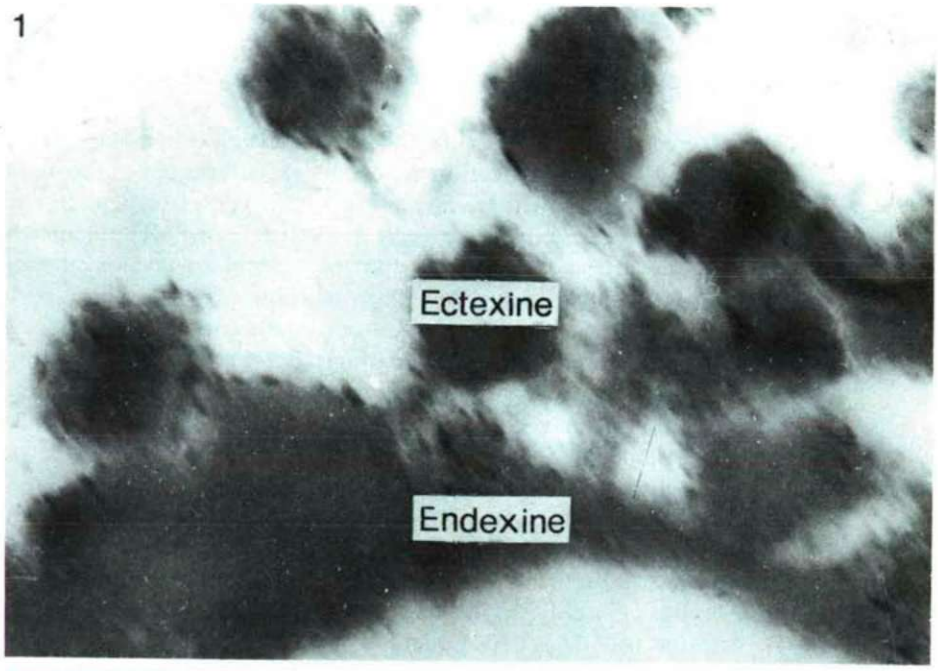
T-4b2 — the same, only the length of time was 5^h.

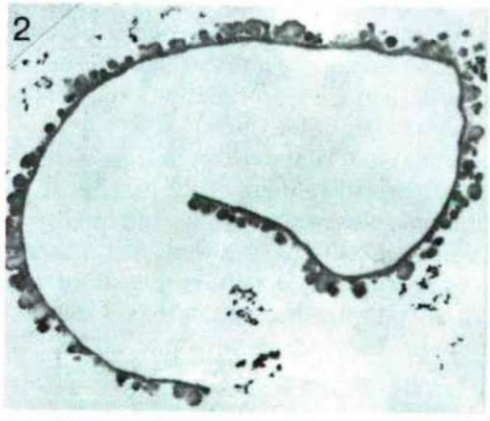
The procedures of the TEM method are identical with the earlier. So after the experiments the pollen material was washed with distilled water, fixed in OsO_4 (aqu. dil.) and embedded in Araldite (Durcupan, Fluka). Ultrathin sections were made on a Porter Blum ultramicrotome with glass knives. TEM pictures were taken in a TESLA BS-500 instrument, which has a resolution of 6 Å.

Plate I

Taxus baccata L.

1. Experiment T-5. Essentially the control infected microbiologically. Well is shown the degradation of the exine, the tiny particules may be microorganisms or the biopolymer units of the sporopollenin. The original lamellar ultrastructure of the endexine may not be discernible. x100000
2. Experiment T-5. General picture from the ultrastructure of the pollen wall. x5000
3. Experiment T-5A; the control of 5^h. The heterogeneous character of the microbial destruction of the exine is well shown on this photomicrograph. The lamellar ultrastructure of the endexine may hardly be recognized, and its electron affinity is stronger than that of the ectexine. x50000
4. Experiment T-5A. In opposition of the previous documentation the lamellar ultrastructure of the endexine is well preserved. Both principal layers of the exine are finely granular, these particules are presumably the globular biopolymer units of the sporopollenin. x100000


1
Ectexine
Endexine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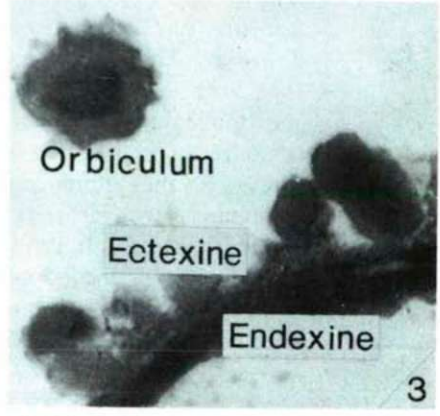
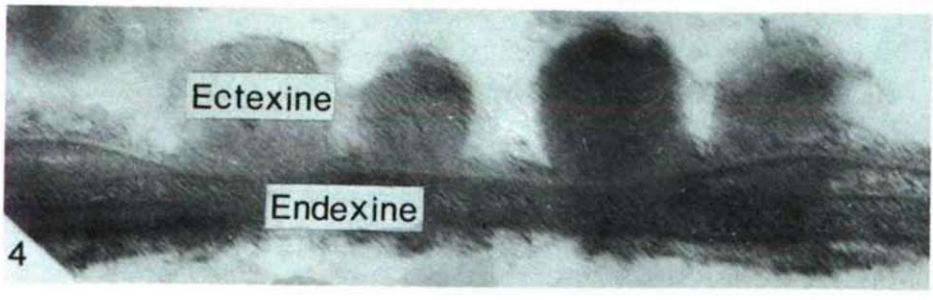

Orbiculum
Ectexine
Endexine
3


Ectexine
Endexine
4

Results

By the different experiments, the most important results may be summarized as follows:

T-5 — This is the control of $2^h30'$, its results are surprising (Plate I, figs. 1,2). The picture of low magnification gives a general aspect about the exine stratification of the pollen grain (Plate I, fig. 2). The picture, with high magnification represents a strongly degraded exine. The lamellar ultrastructure of the endexine may not be recognized on the ectexine there are particules with stronger electron affinity. During the experimental process this material was without doubt contamined, and the exine degraded in this way microbiologically. Concerning the ultrastructure of the non-degraded exine we refer to data published by AFZELIUS (1956) and GULLVAG (1966). The fine structure of the endexine is lamellar, the ectexine is composed of mostly isodiametric particules of various diameter.

T-5A — Essentially the same as previously but there were exines moderately degraded, namely in some cases the lamellar ultrastructure of the endexine was more or less discernible (Plate I, figs. 3,4).

T-7 — (Plate II, fig. 1) and T-7A — The two variants gave identical results. The ectexine was degraded moderately and uniformly; it contrast to this, the endexine dezorganized strongly. The characteristic lamellar ultrastructure of the endexine became homogeneous and partially destroyed. The electron affinity of the ectexine and the endexine at this experiment is identical. In spite of the strong degradation, well defined sporopollenin biopolymer units were not discernible.

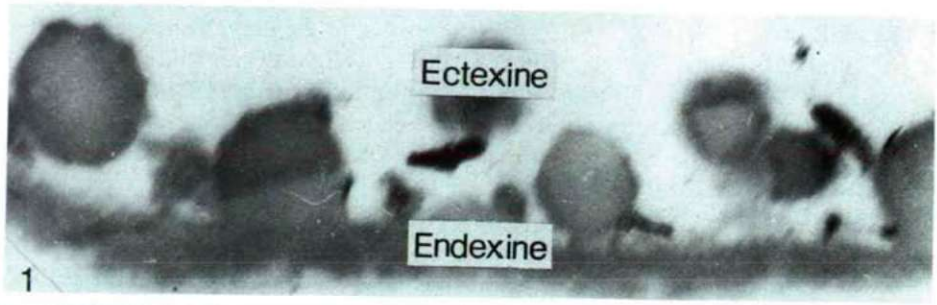
T-9 — (Plate II, fig. 2) and T-9A — The results of these experiments were the same, with the difference that the electron affinity of the homogeneous endexine is stronger in its total thickness, or only in its inner half part (Plate II, fig. 2). It is to be mentioned that the time factor is not considerable in these kind of experiments. It may be presumed that merkapto-ethanol in contrast to the previous results has a peculiar effect.

Plate II

Taxus baccata L.

1. Experiment T-7. The degradation of the exine is in general uniform, the lamellar ultrastructure of the endexine may not be discernible. x50000
2. Experiment T-9. Ultrastructure of a strongly degraded exine. The endexine is secondarily homogeneous, by its electron affinity two sub-layers may be distinguished. The electron affinity of the inner part is much more stronger than those of the outer part, this latter mentioned is identical in this respect with the exine. x50000

3,4. Experiment T-11A. The exine is moderately and uniformly degraded, the original, lamellar ultrastructure of the endexine is discernible in spite of its damaged character. x100000


Ectexine
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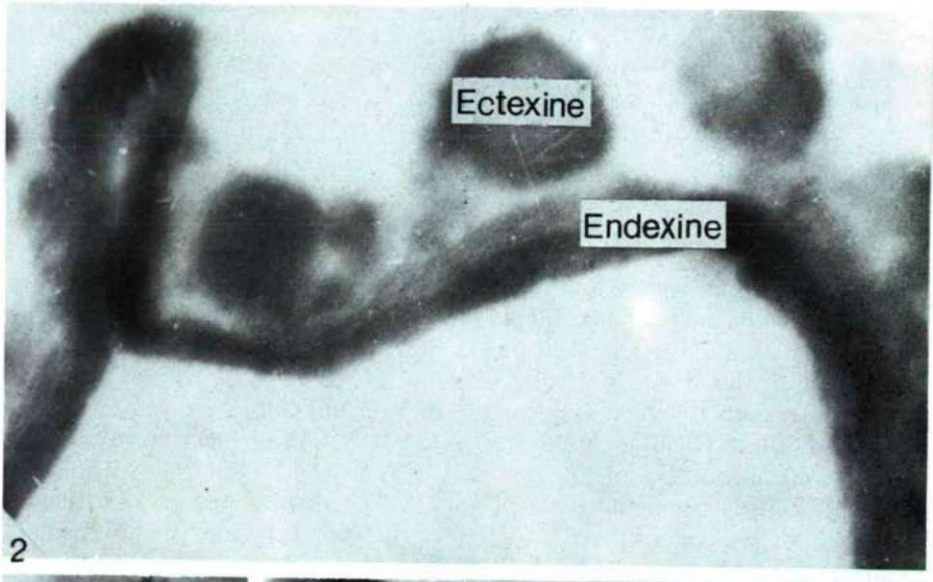
1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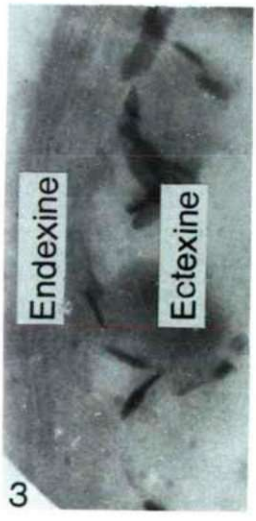
Endexine
Ectexine
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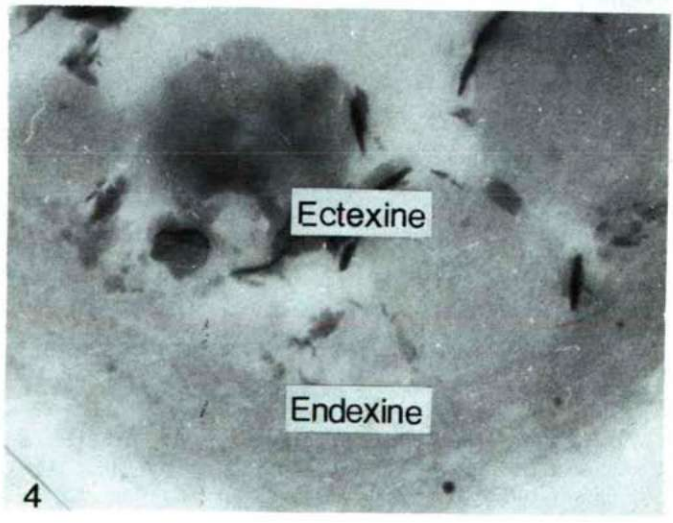

Ectexine
Endexine
4

T-11 and T-11A — (Plate II, figs. 3,4) — In contrast to the previous two experiments (T-7 and T-9) the exine degraded in a slight degree and uniformly. As regards the electron affinity of the different layers of the exine no differences were observable. The endexine was strongly degraded, but its original lamellar ultrastructure was observable on the biggest part of the ultrathin sections.

T-4b1 and T-4b2 — (Plate III, figs. 1-3) — This experiment produced a characteristic degradation. The endexine lost its lamellar ultrastructure, in some places, tiny granules occur (Plate III, fig. 3) which may be the globular biopolymer units of the sporopollenin. The electron affinity of the ectexine and the orbiculi are much more stronger than that of the endexine. Furthermore there are differences in the electron affinity of the wall of the orbiculi, a very narrow, inner layer has a stronger electron affinity than the thicker outer layer.

Discussion and conclusions

1. The experimental degradation methods, which resulted at the exine of *Corylus avellana* in well defined globular biopolymer units in the case of the *Taxus baccata* brought not a similar result. This relate that the chemical composition, and in consequence of this in the molecular structure of the exine of *Corylus* and *Taxus* there are essentially differences. In connection with this it is interesting to cite from the paper of UENO (1960) the following; p. 126/127: "The pigments were studied by SUITA (1948), KARRER and LEUMANN (1952) etc., and LUBLINER-MIANOWSKA (1955) investigated pigments in pollen grains of 67 species. According to him the pigments in pollen grains of conifers is not carotenoid, while that of entomophilous pollen of angiosperms is carotenoids." But BROOKS (1971) established as follows; p. 351: "The chemical study of various modern and fossil spore walls of gymnosperms, angiosperms, pteridosperms fungi and algae show a majority to be composed of sporopollenin.

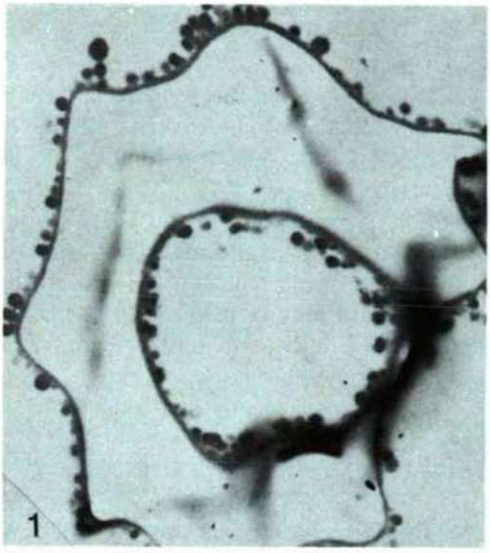
2. The effects of the degrading secrets used during the experiments are different. The *Helix* enzyme strongly degraded the endexine, but have not differentiate the electron affinity of the two principal layers of the exine.

Plate III

Taxus baccata L.

1—3. Experiments T-4b2

1. General picture from the exine ultrastructure of the pollen grain. x5000
2. Fine structure of the degraded orbiculum. x100000
3. Detail from the ultrastructure of the degraded exine. The electron affinity of the orbiculi and the ectexine is stronger than those of the endexine. In consequence of the degradation the lamellar ultrastructure of the endexine may not be discernible. x100000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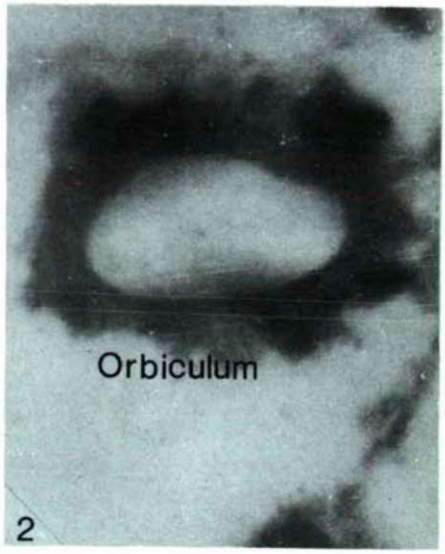
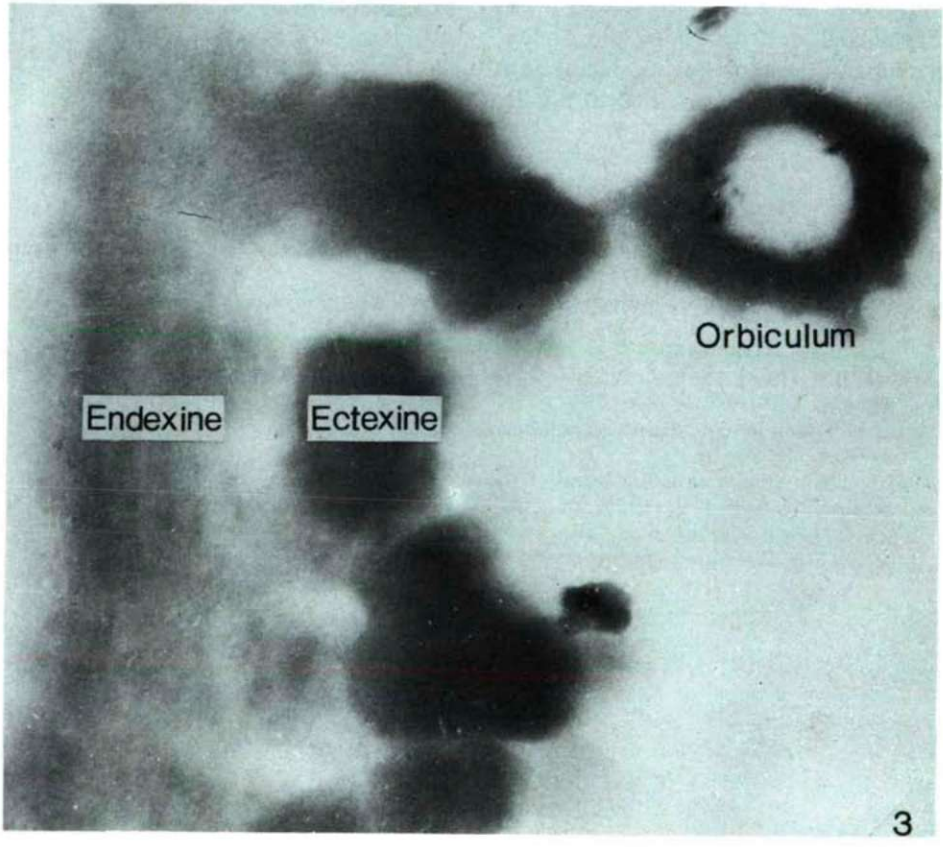

Orbiculum
2


Orbiculum
Endexine
Ectexine
3

3. The merkapto-ethanol cause such changes in the chemical structure which appear in the electron affinity of the two principal layers of the exine. The exclusive use of the merkapto-ethanol resulted that the electron affinity of the ectexine alternate stronger. The merkapto-ethanol, with *Helix* enzyme altered the electron affinity of the exine layers, but caused an opposing effect, so the electron affinity of the endexine became stronger than that of the ectexine.
4. The use of EDTA as a supplement of these experiments, on the basis of our present day knowledge moderate the effect of *Helix* enzyme and merkapto-ethanol.
5. The microbial degradation naturally was not projected, but it is warning concerning the control. On the other hand these data may be also useful in further researches. The experimental microbial degradation is in general another field of the researches of the exine.

ACKNOWLEDGEMENTS

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References

AFZELIUS, B.M. (1956): Electron-microscope investigations into exine stratification. — Grana Palynologica *1*, 22-37.

BROOKS, J. (1971): Some chemical and geochemical studies on sporopollenin. In: Sporopollenin (eds. BROOKS, J., GRANT, P.R., MUIR, M., van GIJZEL, P. and SHAW, G.). — Acad. Press, London — New York, 351-407.

GULLVAG, B.M. (1966): The fine structure of some gymnosperm pollen walls. — Grana Palynologica *6*, 325-475.

KEDVES, M. (1986): In vitro destruction of the exine of recent palynomorphs I. — Acta Biol. Szeged. *32*, 49-60.

UENO, J. (1960): Studies on pollen grains of *Gymnospermae*. Concluding remarks to the relationship between *Coniferae*. — J. Inst. Polytechnics, Osaka City Univ. D, *11*, 109-136.

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MOLECULAR STRUCTURES FROM THE ORGANIC REMNANTS OF THE CARBONATE MANGANESE ORE LAYERS OF THE III. SHAFT OF URKÚT, HUNGARY

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Abstract

The results of the transmission electron-microscope studies on the microfossils of *Pleurozonaria concinna* type extracted from the carbonate manganese ore layers of Urkút are the following: The submicroscopical structure of the wall of these microfossils partially degraded, probably during the sedimentation process, and in consequence of this in some places globular biopolymer units may be recognized. On the surface of the spheric microfossils several particules have been adsorbed. 1. Narrow particules, which adhere closely to the surface of the microfossils, their electron affinity is stronger than that of the wall. 2. Irregular masses, at the places of their contacts the surface of the wall is destroyed. 3. Probably polymer units, which are slack, mostly composed from irregular masses between them helical structures were also observed. The helical structures were described first as higher organized biopolymer units of the sporopollenine of the angiosperm pollen grains.
Key words: Jurassic — manganese ore — *Pleurozonaria* — wall molecular structure.

Introduction

The plant microfossils of the manganese ore layers of Urkút were the subject of several previous publications. Using the light-microscope method the description of the basic microflore assemblage and the reconstruction of the vegetation zones of the coastal area (SIMONCSICS and KEDVES 1961, 1969, KEDVES and SIMONCSICS 1964a,b, 1971). The first transmission electron-microscope investigations on the exine of *Spheripollenites scabratus* COUPER 1958 come to an angiosperm type ectexine, in contrast to the earlier establishment of gymnosperm affinity of this species (KEDVES and PÁRDUTZ, 1973). The first SEM data on the plant microfossils of the carbonate manganese ore layers of Urkút were published by KEDVES and RADVÁNSZKI (1975) from *Pleurozonaria concinna* (COOKSON and MANUM 1960) MADLER 1968. Later KEDVES (1976) described his results on the SEM studies of *Classopollis classoides* (PFLUG 1953) POCOCK and JANSONIUS 1961. In spite of the fact that it was a long time ago when the taxonomical elaborations based on the light-microscope method were published, and there were several changes in the nomenclature, but in this respect there is no serious essential change. But till this time we lack detailed taxonomy and modern study, firstly electronmicroscopical

investigations of the planctonic organisms of the manganese ore layers of the III. shaft of Urkút. This paper is a part of these supplementary results, and summarize the first data on the molecular structures of the wall of the planctonic organisms of the manganese ore layers.

Material and Methods

The material of investigations is sample No 7 of the III. shaft of the manganese ore layers of Urkút, green, greenish brown, light green, grey roughly streaked carbonate manganese ore. The preparations for LM investigations were made in 1963, the embedding for TEM studies, in 1973. TEM pictures were taken on Tesla BS-500 electron microscope in the Electron-Microscope Laboratory of the Faculty of Science of the J.A. University. I express my thanks to Dr. I. ROJIK for his kind help.

It is necessary to emphasize that we have a number of publications concerning the destructive effect of the $KMnO_4$ to the ultrastructure of the plant cells.

The subject of investigations were three different types of *Pleurozonaria concinna*, their block No: 85/6, 85/7, 85/8. Before the ultrathin sections, LM pictures were taken from the specimens of investigations. The detailed elaboration and evaluation of the ultrastructure of the whole planctonic organisms of the manganese ore layers of Urkút needs further investigations.

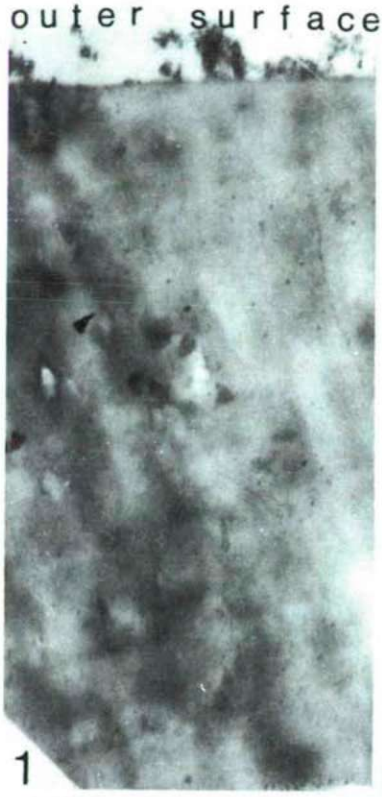
Results

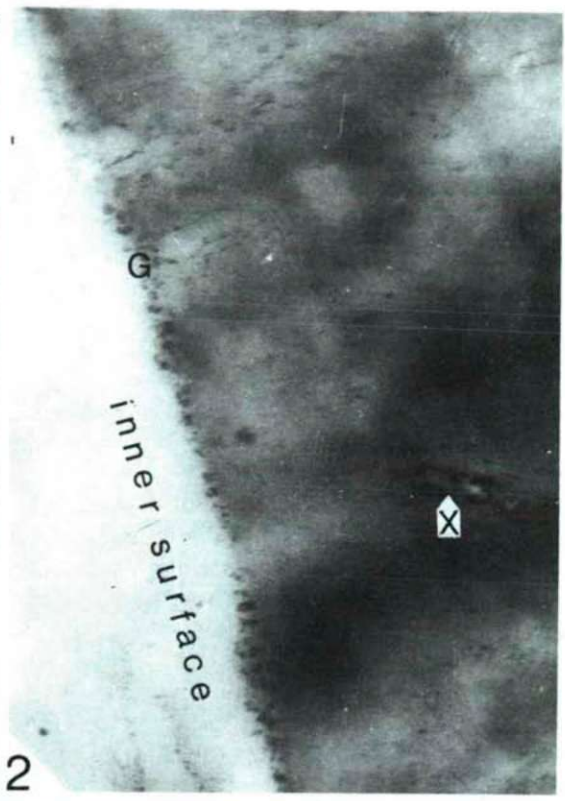
A general view about the ultrastructure of the wall of the planctonic organisms is represented by picture 1, Plate I. Well shown are the hollows oriented in the radial direction of the wall. On the surface there are more or less regularly adsorbed particules. The substance of the wall is originally homogeneous, but in some parts fine granular structures may be recognized. In the right corner of the picture, a part of the inner surface is represented with globular units, which have a relatively strong electron affinity. The same with higher magnification is well shown in fig. 2, of Plate I (G). In all probability the wall of the microplancton organisms was oxidized during the sedimentation. On the inner surface of the hollows of the wall, these globular units are also well shown (X). On the high magnified pictures (Plate I, figs. 3,4) a certain arrangement of these globular polymer units may be established, but our up-to-date knowledge is not enough for the evaluation of this. The diameter of the globular biopolymer units is about 8 Å.

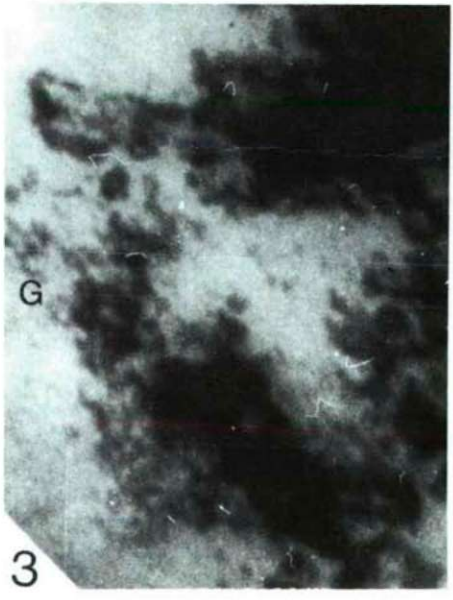
Plate I

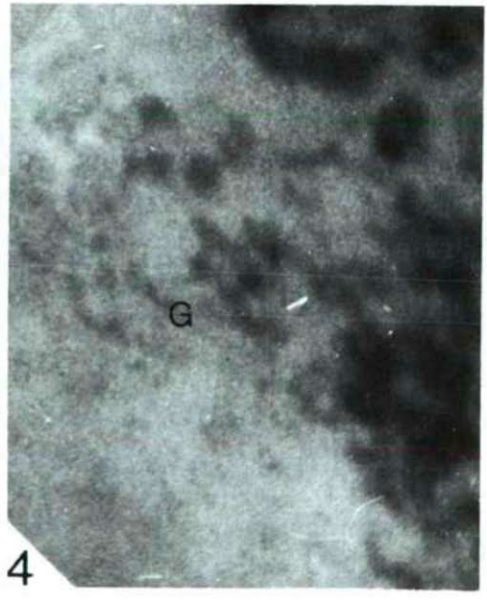
Pleurozonaria concinna (COOKSON et MANUM 1960) MÄDLER 1968

1. Block No: 85/8, cross-section of the wall. x20000
2. Block No: 85/8, the inner surface of the wall. G = globular polymer units on the inner surface, X = polymer units on the surface of the hollow in the wall. x48000
3. Block No: 85/6, globular polymer units of the wall (G). x250000
4. Block No: 85/6, globular polymer units of the wall (G). x500000


outer surface
1


G
inner surface
X
2


G
3


G
4

The origin of the particules adsorbed on the surface of the wall is doubtful, but it seems to be interesting in respect of further investigations. Worth of mentioning are the helical structures (H) on the surface, which occur in a mass. The inner part of the core lack in our helical structures. Moreover there are massulae without structures on the surface of the wall of the planctonic organisms. We have observed the degradation of the wall at the point of contacts with these remains (A). Finally there are narrow particules on the surface, with stronger electron affinity than that the wall of the microplancton organisms.

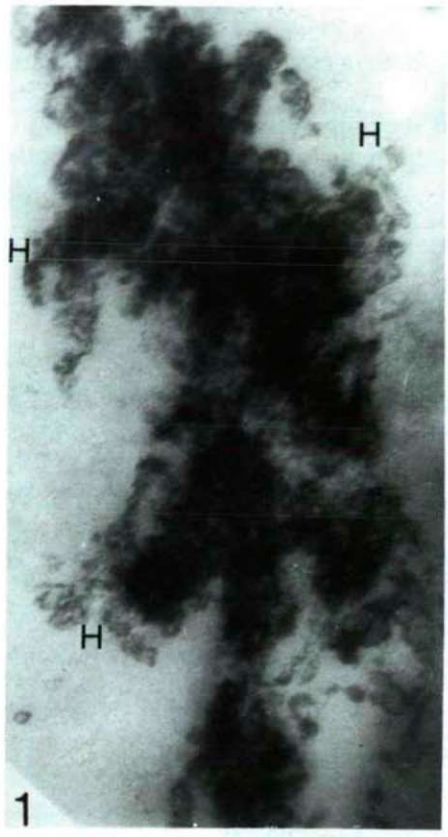
Discussion

1. Our results refer that the molecular structure of the microplancton organisms may be open up partially during the sedimentation process of the manganese ore layers.
2. The observed molecular biopolymer units are globular, and compared with the previous data are relatively small (cf. KEDVES et al.)
3. The exact origin of the particules of three types adsorbed on the surface of the plancton organisms is now in question. These may be refer to microbial, bacterial activity. The helical structures may originate from the organic material of strongly degraded exine of pollen grains. As another opportunity it is possible that these are inorganic remnants of the iron-manganese bacteria.
4. The experimental method, using the oxidation with $KMnO_4$ is necessary for the fossil plant microfossil remnants too. From the literature in connection with the recent pollen grains we stress the importance of the following publications:
ROWLEY, DAHL and ROWLEY (1980); p. 252: "We find the pollen grains exine of *Artemisia vulgaris* to be composed of units 6—100 nm in diameter, consisting of helical subunits about 10 nm in diameter". Methods, p. 252: "Exines of mature pollen grains were oxidized in 2-aminoethanol for 40 sec at 90 °C. Exines were washed free of the organic base in 100nM phosphate buffer at pH 6.5, then kept in freshly prepared 0.01% $KMnO_4$ (phosphate buffered, pH 6.5) for 40 days to oxidize lipids and unmask proteins in glycocalyx elements of exinous subunits. Lipids were extracted in an acetone-epoxy resin series (2) beginning with 20 pts acetone: 1 pt epon-araldite."

Plate II

Pleurozonaria concinna (COOKSON et MANUM 1960) MÄDLER 1968

1. Block No: 85/6, particules adsorbed to the surface with helical units = H. x120000
2. Block No: 85/6, helical units adsorbed to the surface with higher magnification. x250000
3. Block No: 85/7, the outer surface of the wall with different kind of adsorbed particules, A, B. One part of the hollow in the wall well shown. x64000


H
H
H
1


H
2


B
A
3

ROWLEY (1980), p. 358: "Our model of the exinous unit and its subunits (Fig. 1) has a super-coiled binder around one to many core subunits." Reviews about this subject: ROWLEY, DAHL and ROWLEY (1981), ROWLEY, DAHL, SENGUPTA and ROWLEY (1981).

5. Finally, the comparison and evaluation of the molecular structures which were open up under natural and in vitro circumstances is a new opportunity to the knowledge of the conditions of the sedimentation process of the manganese ore layers.

References

KEDVES, M. (1976): Scanning electron-microscopic investigations on the pollen grains of the *Operculati* VENK. et GÓCZ. 1964. — Acta Biol. Szeged. *22*, 29-36.

KEDVES, M. and PÁRDUTZ, Á. (1973): Ultrastructure examinations of fossil *Pteridophyta* spores and *Gymnospermatophyta* pollens. — Acta Bot. Acad. Sci. Hung. *18*, 307-313.

KEDVES, M. and RADVÁNSZKI, M. (1975): The application of scanning electron microscopical method in some plant microfossils. — Acta Bot. Acad. Sci. Hung. *21*, 51-59.

KEDVES, M. and SIMONCSICS, P. (1964a): Microstratigraphy of the carbonate manganese ore layers of the shaft III of Urkút on the basis of palynological investigations. — Acta Miner.—Petr. *16*, 3-48.

KEDVES, M. et SIMONCSICS, P. (1964b): Spores nouvelles extraites de minerai de manganèse Jurassique de la Région d'Urkút (Hongrie). — Pollen et Spores *6*, 605-610.

KEDVES, M. and SIMONCSICS, P. (1971): Investigation of spores and pollen grains of the carbonate manganese ore bore samples from Urkút. —Acta Miner.—Petr. *20*, 85-96.

KEDVES, M., STANLEY, E.A. et ROJIK, I. (1974): Observations nouvelles sur l'ectexine des pollens fossiles des Angiospermes de l'Eocène inférieur. — Pollen et Spores *16*, 425-437.

ROWLEY, J.R. (1981): Pollen wall characters with emphasis upon applicability. — Nord. J. Bot. *1*, 357-380.

ROWLEY, J.R., DAHL, A.O. and ROWLEY, J.S. (1980): Coiled construction of exinous units in pollen of *Artemisia*. — 38th Ann. Proc. Electron Microscopy Soc. Amer., 252, 253.

ROWLEY, J.R., DAHL, A.O. and ROWLEY, J.S. (1981): Substructure in exines of *Artemisia vulgaris*. — Rev. Palaeobot. Palynol. *35*, 1-38.

ROWLEY, J.R., DAHL, A.O., SENGUPTA, S. and ROWLEY, J.S. (1981): A modell of exine substructure based on dissection of pollen and spore exines. — Palynology *5*, 107-152.

SIMONCSICS, P. and KEDVES, M. (1961): Paleobotanical examinations on manganese ore series in Urkút (Hungary, Transdanubia). — Acta Miner.—Petr. *14*, 27-57.

SIMONCSICS, P. and KEDVES, M. (1969): The pollen grains of the carbonate manganese ore of the shaft III. in Urkút. — Acta Miner.—Petr. *19*, 85-94.

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THE VEGETATION MAP OF THE KISAPAJ UNESCO BIOSPHERE RESERVE CORE AREA, KISKUNSÁG NATIONAL PARK, HUNGARY

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Abstract

The paper comprises the vegetation map, on a scale of 1:5000, of the Kisapaj UNESCO biosphere reserve core area which is under increased protection.

The majority of the core area is covered by halophilic plant communities, with smaller of sandy *(Astragalo-Festucetum rupicolae, Potentillo-Festucetum pseudovinae)* and loess *(Salvio-Festucetum rupicolae)* associations, which also become halophilic, to a larger or lesser extent. The variegated microrelief, as well as the two meters' grade difference between the highest and deepest reliefs have promoted the development of a vegetation variedness, expanding from the *Salvio-Festucetum rupicolae* to *Caricetum acutiformis-ripariae* associations, along the environmental gradient(s) defined by the groundwater level. The most characteristic association are: the *Salvio-Festucetum rupicolae, Achilleo-Festucetum pseudovinae, Artemisio-Festucetum pseudovinae, Agrostio-Alopecuretum pratensis, Caricetum acutiformis-ripariae.* In the present report, the cenological characterization of these associations is given at a depth necessary for the interpretation of the units on the vegetation map.

Key words: aerial photograph, biosphere reserve, environmental conservation, halophilic vegetation, vegetation mapping

Introduction

The thorough phytogeographical-floristic exploration of the northern part of the Kiskunság (a district in South-Central Hungary) was started after the establishment of the Kiskunság National Park (KNP) (TÓTH, 1985). It is interesting that the early floristic studies on the halophilic vegetation in Kiskunság were carried out more intensively in the southern regions that in the northern area, which is almost in the immediate neighbourhood of Budapest. Elaborate reports are given by RAPAICS (1927) from the environs of Szeged and by PRODÁN (1914) about the alkali plains in the former county of Bács-Bodrog. Regarding the evaluation of vegetational changes taking place in the mapped area, the paper of MOESZ (1940) is supplementary and of great importance. The publication enables conclusions to be drawn as to the vegetation in the Kunszentmiklós-Apaj area at that time (in 1929), by means of precise position designations used to compare the alkali plains of the Northern Kiskunság and the Jazygia.

The documentation on the present state of vegetation is necessitated because of the increased endangerment of the area, owing to the scant number of publications, greater protection, and direct and indirect anthropogenic effects (water management and grazing). The first step means the preparation of a vegetation map, as a map of similar precision has not yet been published about the area.

Materials and methods

The vegetation map was prepared on the basis of a black and white aerial photograph, the magnification of which corresponded to a map on scale 1:5000. A great advantage of vegetation maps prepared by means of aerial photographs is that their preciseness, and fullness of details surplass those of maps prepared on the ground. The unevenness of the field, as well as the relief and microrelief do not cause problems during the course of preparation (JAKUCS, 1966). An aerial photograph reveals many details which are imperceptible and unseen close to the surface. The stands of the vegetation units are detectable in varying shades on a black and white aerial photograph, mostly in connection with the vegetation coverage. On salt-affected (light) soils communities of high coverage are seen to be dark. The labellings on the vegetation map were chosen on the basis of the intensity of shades observable on the aerial photograph. With the shades of identical intensity, the varying consistence of the shades on the aerial photograph (blades, homogeneity, patchiness) also serves to delineate the different communities. Despite this, the extension of objects which are difficult to differentiate on the photograph should be checked by means of measuring with a tape-line on the spot. The stands of the communities, and their units within the community, of the most characteristic and adequately large surface (larger in area than five metres) found in the region are indicated on the vegetation map. A detailed cenological review of these would require an analysis greatly beyond the possible extent of this paper — due to the rather compliceated transitional character of the communities, often even being of a degradative nature. The preliminary mapping was accomplished in 1984, the detailed map was elaborated in 1985.

In approximately a north-south direction, a 30—40 cm deep ditc — which has long lost its function — runs through the area; this is indicated on the map by thick dotted line.

The map is issued in the form of sheets joining without overlap. The four map sheets (A, B, C, D) are formally published as an appendix to this paper. The contiguous combination of the sheets can be clarified on the basis of Fig. 1.c., indicating the most characteristic community boundaries. The scale of the sheets is 1:5000: The explanations of the labellings on the map are given in Fig. 2. The boundaries of the vegetation units, which are easily distinguisable in the region are indicated on the map by a continuous line, and the boundaries of the vegetation units forming transitions to each other, but not sharp community limits, by dotted line (cf. SIMON, 1957).

Results

GENERAL CHARACTERIZATION OF THE AREA

The mapped core area is situated in the "Kiskunság alkali plain", II. area of the KNP (Fig. 1. a,b.) Three biosphere reserve core areas have been demarcated in the II. area of the KNP, from which the area between Apaj and Kunszentmiklós is the mapped Kisapaj core area (Fig. 1. b,c.). This core area, just as the entire II. area of the KNP, belonged to the flood area of the Danube prior to its controlled in the 1870s. Its relief bears all the marks of the inundations of the Danube (PÉCSI, 1959).

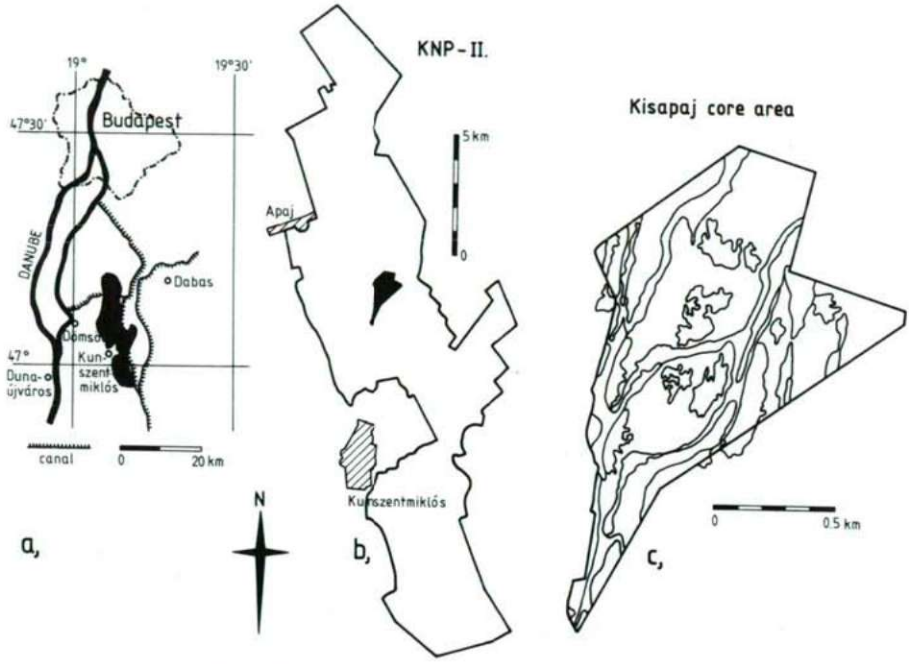

Fig. 1. The geographical localization of the Kisapaj UNESCO biosphere reserve core area

The water receding after the inundations established streamlets, mostly in a north-south direction, which connected to each other, form a complicated network. Saline ridges are found between them, and the larger ridges have become cultivated plough-land. These are located on the border of the core area. Since these parts of high relief are not large within the core area, they are not suitable for plough-land cultivation. Thus, being unaffected by most drastic anthropogenic impacts, a "natural-like" vegetation has developed and still maintained here.

The heterogeneity of the microrelief has led to the mosaic-like nature of the vegetations. In case of salt affected, sodic, alkaline or saline soils, the development of the various associations is determined by the relief, under given climatic conditions — by means of the influence on the groundwater depth, hereby on the trend of salt and carbonate migration processes, and on the possibilities of organic matter accumulation (VÁRALLYAY and MIRONENKO, 1979; BRESLER et al. 1982).

A BRIEF CENOLOGICAL CHARACTERIZATION OF THE VEGETATION FOUND IN THE AREA

The description of the vegetation in the core area is given only to the depth necessitated for the interpretation and evaluation of the vegetation units indicated on the vegetation map. The nomenclature of SOÓ (1964) is used for the designation of the cenosystematic units found in the paper, taking into consideration the subsequent changes in name of the species.

1. *Salvio (nutanti-nemorosae)* — *Festucetum rupicolae* ZÓLYOMI 58.
Salvio nemorosa — *Festuca rupicola* loess meadow association

Dominating species: see below.

This cenological name sums up the vegetation found in the highest parts of the area. The name actually covers the complicated mosaic complexes of the association mentioned here, as well as of the *Astragalo-Festucetum rupicolae*, *Potentillo-Festucetum pseudovinae*, and *Cynodonto-Poetum angustifoliae* associations developing on the effect of anthropogenic impacts (drying up as a consequence of ground canalization, grazing, and disturbing the soil), and also of the transitional stands of these associations.

The complexity, mosaic-like nature of the associations can be traced to pedological causes. First of all, the *Salvio-Festucetum rupicolae* loess association did not develop here on typical loess soil, but on clayey alluvial soil, being rather similar to loess — as regards the composition of the grain size. This layer is rather thin, gradually being replaced, by sand in a downward direction. As a consequence, sandy associations may develop on it as an effect of the slightest erosion, or soil disturbance: *Astragalo-Festucetum rupicolae*, *Potentillo-Festucetum pseudovinae*. Sand may settle on this layer; even this case, the *Astragalo-Festucetum rupicolae* association can be found on it. These association-fragments cannot be distinguished on a map drawn on a given scale.

The *Salvio-Fastucetum rupicolae* unit indicated on the map gives a summary of those stands in which the following *Festucion rupicolae*, *Festucetalia valesicae*, *Festuco-Brometea* species occur with a high coverage and frequency: *Asperula cynanchica*, *Astragalus asper*, *Astragalus austriacus*, *Bothriochloa ischaemum*, *Coronilla varia*, *Festuca rupicola*, *Filipendula vulgaris*, *Galium pedemontanum*, *Galium verum*, *Hieracium bauhini*, *Hiearcium pilosella*, *Koeleria cristata*, *Linum austriacum*, *Medicago falcata*, *Potentilla recta*, *Salvia nemorosa*, *Scabiosa ochroleuca*, *Stipa capillata*, *Thymus glabrescens* and *Verbascum phoeniceum*. From the characteristic species of the great coverage of other cenosystematic groups, the *Carduus nutans*, *Cynodon dactylon*, *Dactylis glomerata*, *Euphorbia cyparissias* and *Ononis spinosa* are typical.

The *bothriochloetosum ischaemi* and *stipetosum capillatae* units — indicated as subassociations — can be distinguished in the region relatively easily. In case of these, the predominant coverage of the denominative species, and the lower abundance and diversity of species (facies character) as compared to a typical

association, are characteristic (cf. SOÓ 1964). The degradative character of the *bothriochloetosum* is not doubtful here (VIRÁGH and FEKETE, 1984).

2. *Achilleo — Festucetum pseudovinae* (MAGYAR 28) SOÓ (33) 45. *Achillea collina — Festuca pseudovina* alkali plain grassland association

Dominating species: *Achillea collina, Alopecurus pratensis, Artemisia santonicum, Cynodon dactylon, Festuca pseudovina, Inula britannica, Plantago maritima, Podospermum canum.*

The *Achilleo-Festucetum pseudovinae* category indicated on the map sums up the following units within the association, adopted to the various hydroecological and salt conditions: *alopecuretosum* and *artemisietosum* subassociations and the *Plantago maritima* variant. Typical *Achilleo-Festucetum pseudovinae* stands occur mainly on the higher reliefs; in case of maps drawn to a given scale, they cannot be illustrated in an exact manner separated from the communities indicated on these reliefs.

3. *Artemisio — Festucetum pesudovinae* (RAPCS. 16, MAGYAR 28) SOÓ (33) 45. *Artemisia santonicum — Festuca pseudovina* wormwood alkali plain association

Dominating species: *Artemisia santonicum, Festuca pseudovina, Limonium gmelinii, Matricaria chamomilla, Poa bulbosa, Plantago maritima, Podospermum canum, Puccinellia limosa, Trifolium campestre.*

This association has the greatest extension in the core area. It has almost completely lost its features of origination from the *Lepidio-Puccinellietum limosae*, association being the dominating one in the area before the establishment of the drainage system (MOESZ, 1940). The majority of the *Artemiso-Festucetum pseudovinae* associations form typical stands.

In an essentially smaller area, under favourable relief conditions from the viewpoint of the existence of the *Puccinellia limosa*, the intermediate phases of the *Lepidio-Puccinellietum limosae — Artemisio-Festucetum pseudovinae* transition are observable: i.e. *puccinellietosum* and *artemisietosum* subassociations.

Other *Artemisio-Festucetum* units of a strongly degradative character refer to a connection with the vegetations of the higher reliefs; the *Agropyron repens* facies might be the result of the degradative process of *Astragalo-Festucetum rupicolae — Cynodonto-Poetum angustifoliae*. By means of the organic matter accumulation in its soil, the *Bromus mollis* variant presumably "prepares" the expansion of the communities found on higher reliefs (CLEMENTS, 1916; ref. FEKETE, 1985). In the upper 10 cm layer of its soil segment, the organic matter content is 3.5%, as opposed to the 2.0% humus content of a typical *Artemisio-Festucetum pseudovinae* (based on 5—5 samples).

4. *Agrostio — Alopecuretum pratensis* SOÓ (33) 47. *Agrostis stolonifera — Alopecurus pratensis* meadow foxtail sodic grassland association

Dominating species: *Agrostis stolonifera, Alopecurus pratensis, Inula britannica, Lysimachia nummularia, Rorippa sylvestris* ssp. *kerneri, Scutellaria hastifolia, Taraxacum officinale.*

Its coverage in the area is significant. In a typical floristic composition, it develops on deeper reliefs bordering the *Caricetum associations*. On higher reliefs the xeroseries members adapted to dryness can be distinguished in this association, too. This is caused by the drying out of the soil due to water management. Differential species are the *Agropyron repens*, the *Poa angustifolia*, the *Carex stenophylla* (BODROGKÖZY, 1970). As a collective designation, these are indicated on the map under the name of agropyretosum repentis, the most characteristic and most frequent subassociation.

5. *Caricetum acutiformis-ripariae* SOÓ (27) 30. *Carex acutiformis — Carix riparia* sedgy marshy-meadow association

Dominating species: *Carex acutiformis, Carex disticha, Carex melanostachya, Carex vulpina, Glyceria maxima, Iris pseudacorus, Lysimachia nummularia, Lythrum salicaria, Phragmites australis, Schoenoplectus tabernaemontani.*

The deepest reliefs of the core area are covered by *Caricetum* associations. The majority of these belong to the *Caricetum acutiformis-ripariae* association and its *caricetosum acutiformis* subassociation. The *Caricetum melanostachyae (Caricetum acutiformis-ripariae caricetosum melanostachyae), Caricetum distichae, Caricetum vulpinae association-fragments* covering small areas and having a mosaic-like appearence are illustrated as a part of these units. On the deepest reliefs, where *Caricetum elatae* associations were presumably present, about 30 — 40 years ago, the stands of the *Caricetum acutiformis-ripariae caricetosum ripariae* subassociations are found today. The subassociation is distinguished by the appearance of the *Glyceria maxima* and the *Typhoides arundinacea*, with high coverages.

Further studies

The vegetation map is a source for further studies of the core area. The enumeration already comprising about 300 species is still to be processed and complated. Detailed cenological processing is extremely important from the viewpoint of demonstrating anthropogenic impacts. Several sign refer to the degradative process of the vegetation at Kisapaj: on the higher reliefs the subassociation of the *Salvio-Festucetum rupicolae bothriochloetosum*, the *Bromus mollis* variant of the *Artemisio-Festucetum pseudovinae*, the reed formation in case of the *Caricetum associations*, and the decrease in the individual number of sensitive, but characteristic species, tending towards a value close to extinction. The main, hardly demonstrable danger is caused by the processes developing as an effect of water management, leading to the characterless state of the vegetation. Knowledge of the stages of these processes can be gained by means of studies pertaining to the interrelationship between the soil and the vegetation, for which the detailed cenological processing of the vegetation is indispensable. Parallel to this are measurements regarding the soil data as background factors. Studies should and can be performed with respect to both the general processes valid in case of every community (diversity- and pattern changes, niche segregation) and the manifestation of the

general effects in the various communities. From this point of view, the core area at Kisapaj, with its extremely heterogeneous vegetation, could be an ideal study site.

Only knowledge of the disadvantageous changes in background factors causing the degradation of the vegetation, and the termination of these by means of an appropriate management programme can ensure the maintenance of such characteristic alkaline plains of the Pannonicum — one of the most beautiful and most variegated representatives of which is the Kisapaj UNESCO biosphere reserve core area.

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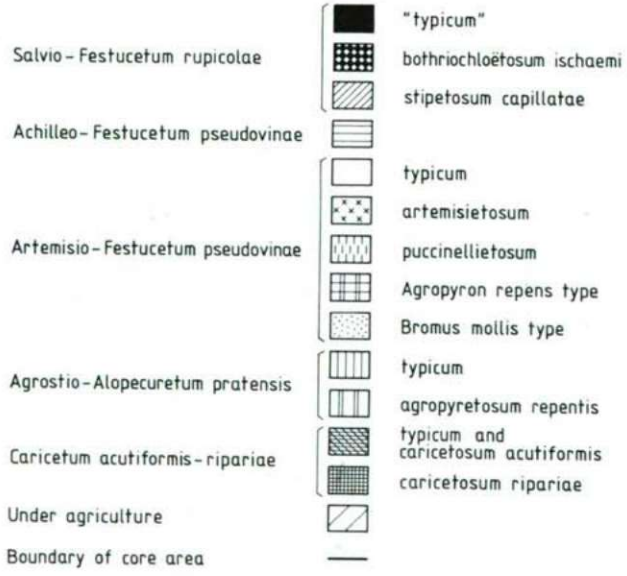
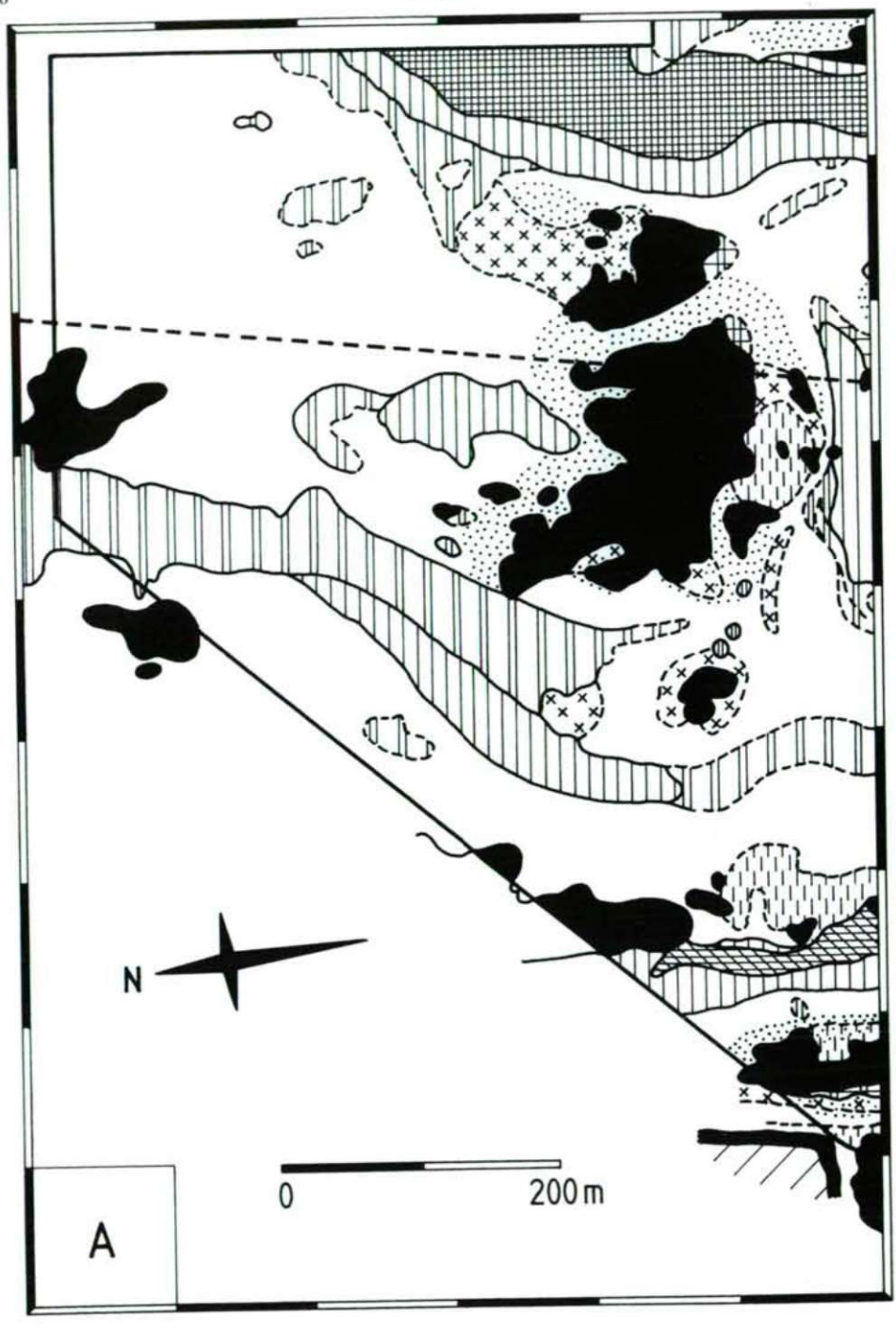


Fig. 2. Key to the signs used in the vegetation map of the Kisapaj UNESCO biosphere reserve core area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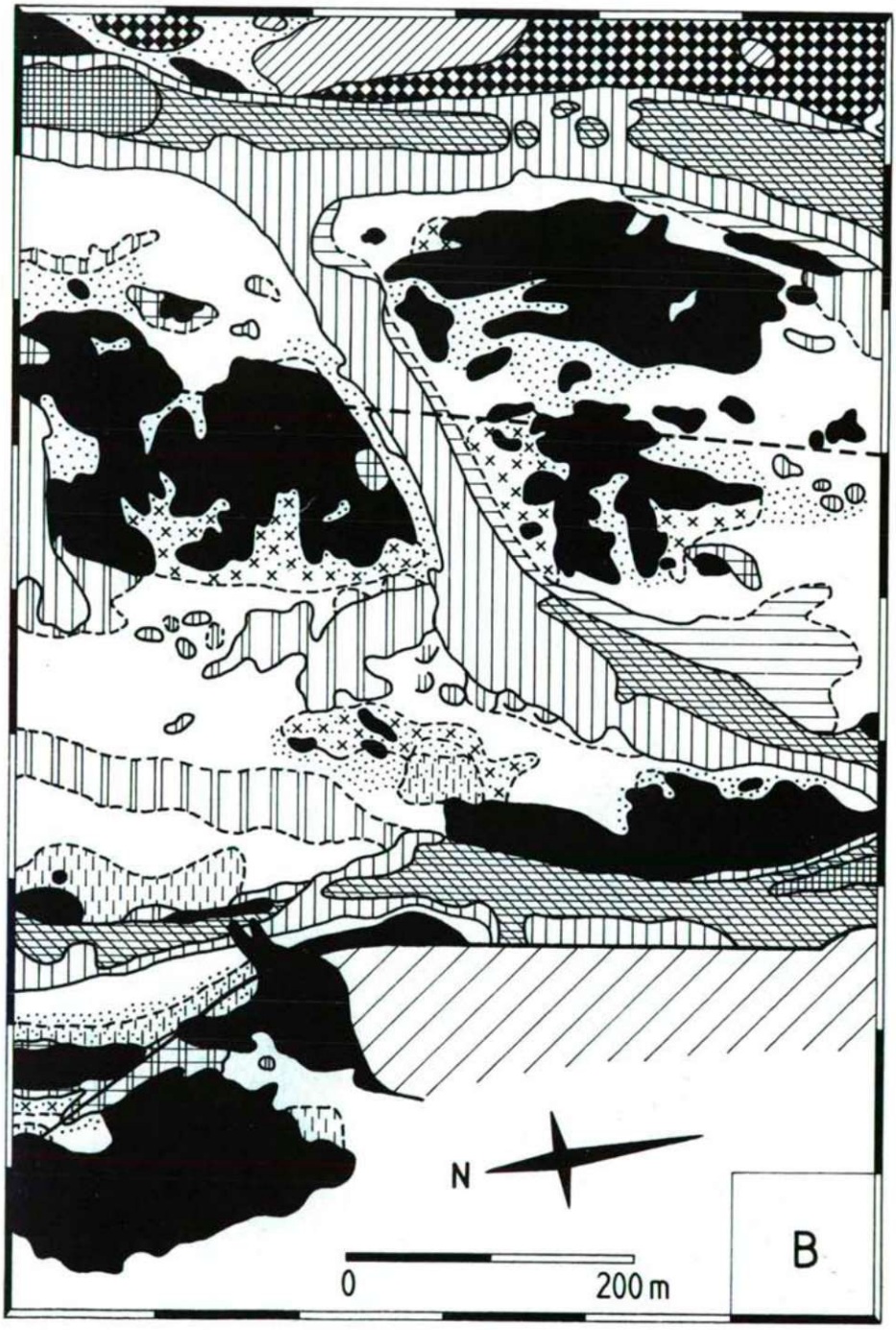
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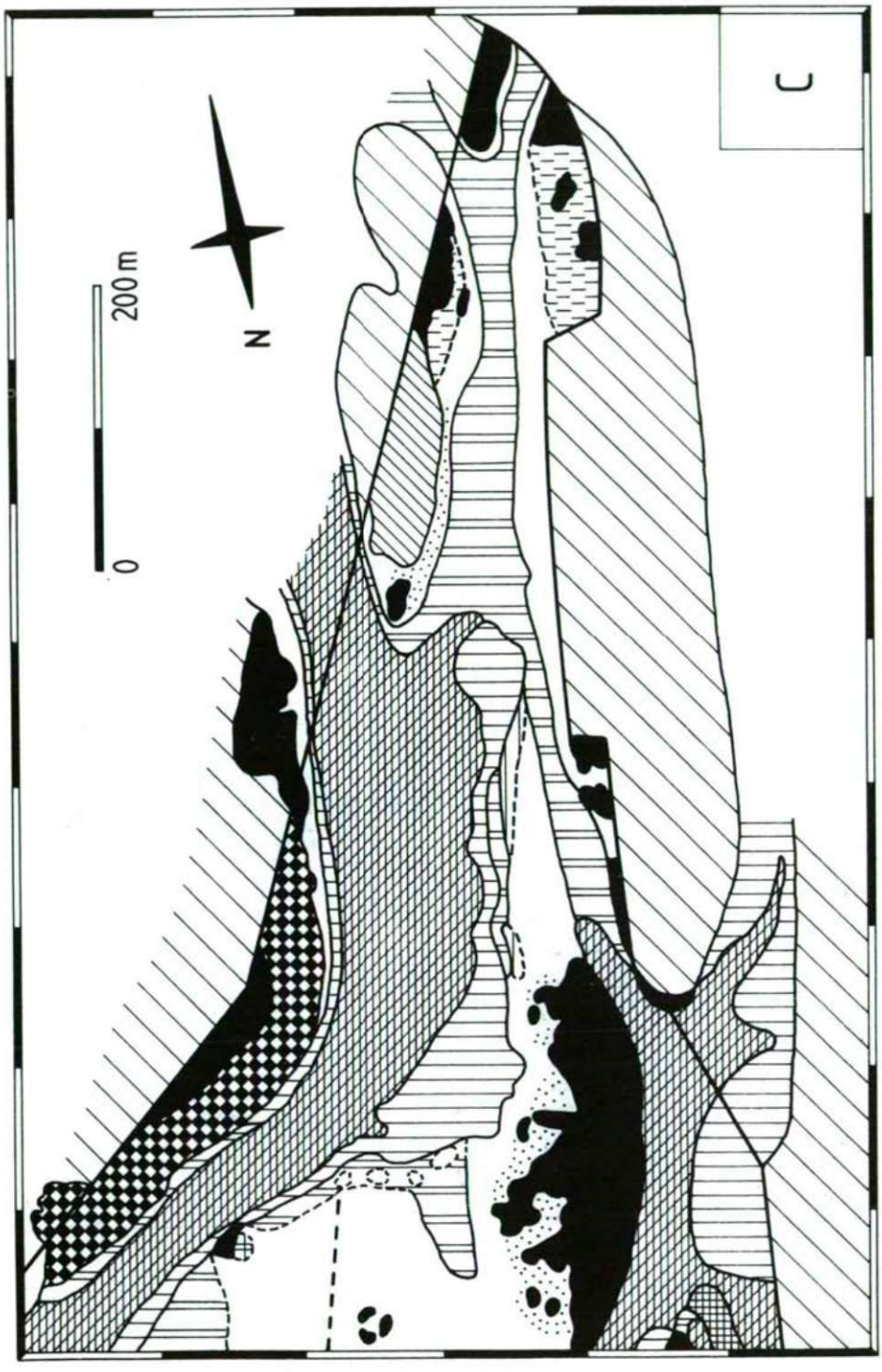
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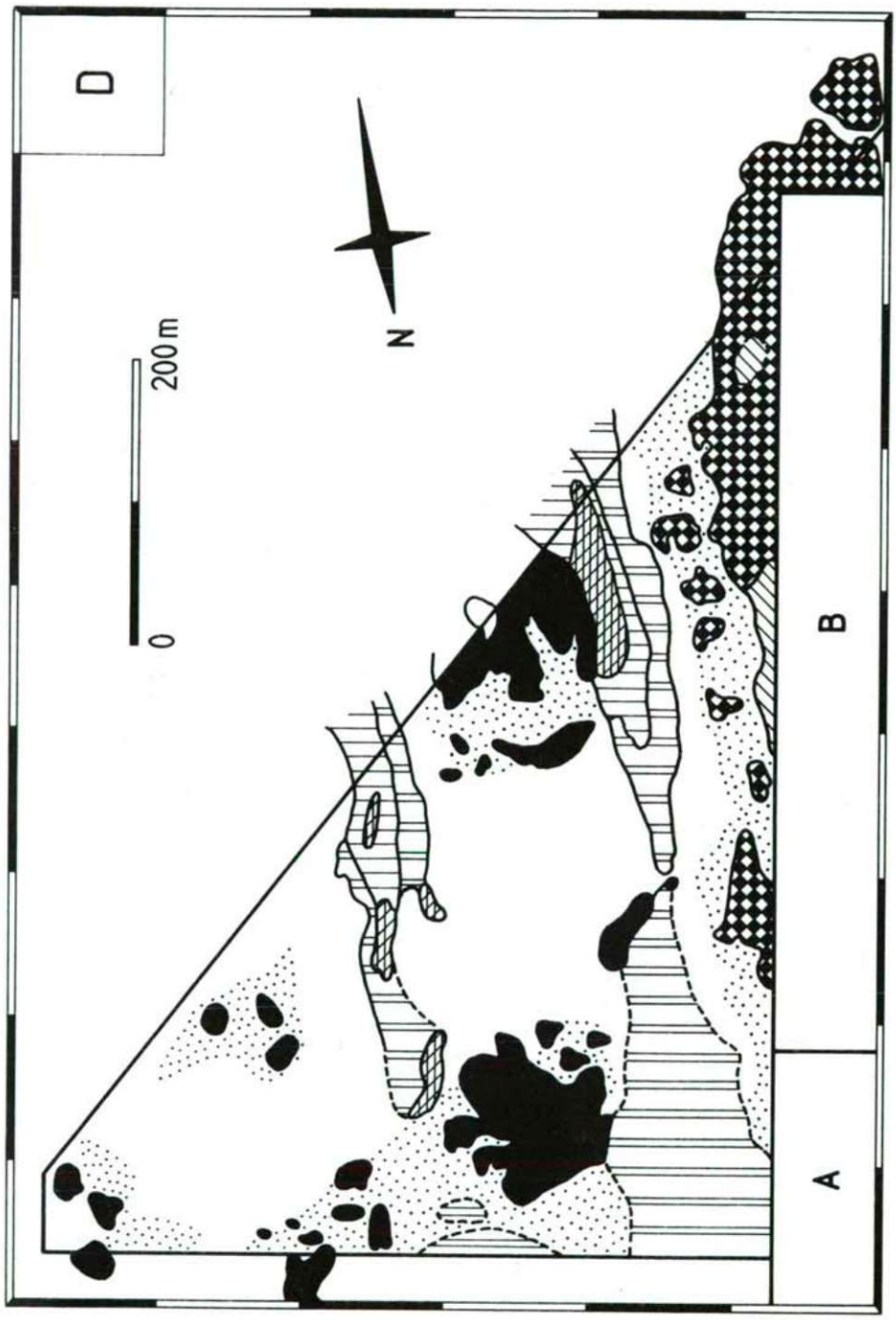
D
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References

BODROGKÖZY, GY. (1970): Ecology of the halophilic vegetation of the Pannonicum VI. Effect of the soil-ecological factors on the vegetation of the reserve of the lake "Dongér" at Pusztaszer. — Acta Biol. Szeged. *16*, 21-41.

BRESLER, E., MCNEAL, B.L. and CARTER, D.L. (1982): Saline and sodic soils. — Springer Verlag, Berlin—Heidelberg—New York.

CLEMENTS, F.E. (1916): Plant succession: an analysis of the development of vegetation. — Carnegie Inst. Washington Publ. *242*, 1-512.

FEKETE, G. (1985): A terrestris vegetáció szukcessziója: elméletek, modellek, valóság (Succession of grassland vegetation: theories, models, facts. In Hungarian). — Biológiai Tanulmányok, *12*, 31-63.

JAKUCS, P. (1966): Légifénykép alapján történő vegetációtérképezés Magyarországon, a Badacsony-hegy példáján (Vegetationskartierung in Ungarn auf Grund von Luftbildern, am Beispiel des Badacsony-Berges). — Bot. Közlem. *53*, 43-47.

MOESZ, G. (1940): A Kiskunság és a Jászság szikes területeinek növényzete (Die Pflanzendecke der Alkalisteppen der Kiskunság und der Jászság). — Acta Geobot. Hung. *3*, 100-115.

PÉCSI, M. (1959): A magyarországi Duna-völgy kialakulása és felszínalaktana (Entwicklung und Morphologie des Donautales in Ungarn). — Akadémiai Kiadó, Budapest.

PRODÁN, GY. (1914): Bács-Bodrog-vármegye sziki növényei (Die Halophytenflora des Komitates Bács-Bodrog). — Magyar Bot. Lap. *13*, 96-138.

RAPAICS, R. (1927): A szegedi és csongrádi sós és szikes talajok növénytársulásai (Die Pflanzengesellschaften der Salz- und Szikböden von Szeged und Csongrád). — Bot. Közlem. *24*, 12-29.

SIMON, T. (1957): Az Északi-Alföld erdői (Die Wälder des Nördlichen Alföld). — Akadémiai Kiadó, Budapest.

SOÓ, R. (1964): A magyar flóra és vegetáció rendszertani-növényföldrajzi kézikönyve I. (Synopsis systematico-geobotanica florae vegetationisque Hungariae I.). — Akadémiai Kiadó, Budapest.

TÓTH, K. (1984): Tudományos kutatások a Kiskunsági Nemzeti Parkban 1975—1984. In Hungarian with English summaries). — OKTH Kiadvány, Budapest.

VÁRALLYAY, GY. and MIRONENKO, E.V. (1979): Soil-water relationships in saline and alkali conditions. — Agrokémia és Talajtan, *28*, Suppl. 33-82.

VIRÁGH, K. and FEKETE, G. (1984): Degradation stages in a xeroseries: composition, similarity, grouping, coordination. — Acta Bot. Hung. *30*, 427-459.

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PATTERN ANALYSIS: A REVIEW AND SOME PROPERTIES OF A METHOD

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Abstract

A wide variety of information can be gained on the spatial pattern of the individuals within a population with the help of the grid analysis developed by GREIG-SMITH. Though the statistical testability of the method is rather weak owing to the dependence of the samples, it is less time consuming than other methods, which is an advantage.

Following the detailed description of the method, a demonstration is given in respect to a few characteristics of the pattern analysis, using a computer program. Following the questions related to aggregation square size and the detectability of the intensity of group formation, the problems pertaining to aggregate orientation are discussed. Based on the one-way complete reduction of the fundamental units of the quadrat grid, the specific spatial orientation of the aggregates is demonstrable.

At the end of the paper, a concrete example is given for the course of the calculation and the mode of evaluation of the analysis.

Key words: pattern analysis, spatial orientation

Introduction

In real space the individuals of a population may show rather varied arrangement, spatial pattern depending on the heteromorphy of the topography, the space-gaining strategy of the population, as well as on the group characteristics of the coexisting populations. Further combinations are possible apart from the familiar three basic types of this pattern (random, clumping, and uniform arrangements), and these frequently complicate the recognition of the real pattern.

Several method have been elaborated for the detection of the spatial distribution of a population (e.g. mathematic distribution functions, distance methods, variance/mean value indices; for details see GREIG-SMITH, 1983). These methods provide authentic information as to the quality of the distribution, but the result is greatly influenced by the unit size of the sample. Furthermore, the scale of the pattern is also left unrevealed. Nevertheless, these methods have good statistical probes. The methods showing pattern scale as well, however, cannot be tested so reliably due to the dependence of the samples.

One of the methods based on the variance of the samples is the grid-analysis developed by GREIG-SMITH (1952), with the help of which the scale and intensity of

the spatial pattern of the populations can also be obtained. (Using the expression "pattern" in the sense given by PRÉCSÉNYI et al. 1967: "by pattern a significant non-randomness is expressed"). Naturally several patterns can be discovered simultaneously in a biocenosis. Characteristic pattern is shown by the vertical and horizontal structure of the communities, by the regulation of the dynamics of the populations living together, etc. (PRÉCSÉNYI, 1981). The present paper only deals with the spatial distribution pattern of the individuals belonging to one single population.

The significance test of the method was elaborated by THOMPSON (1958), the confidence limits were also given. This author also pointed out that contrary to chance pattern arrangement, the orientation of the pattern is also given by the grid-analysis. This property of the analysis was emphasized by PRÉCSÉNYI et al. (1967), too.

Summary of the effectuation of the grid-analysis

Such quadrat grid is to be used for pattern analysis performed with contiguous quadrat grid where the number of basic quadrats, K, is:

$$K = 2^n \ (n = 1,2,3,....) \qquad (1)$$

The grid may be square, rectangle, or a so-called transection.

The elemental squares of the grid containing 2^n squares are regarded as functional unit. The individual number of the studied population (or the presence if the elemental square is small enough to be commensurate with the individuals of the studied species) is recorded in every elemental square during the course of the topometry. This kind of sampling is less time consuming than e.g. the distance

Table 1. 98%, 95% and 90% confidence limits for distribution
(DF = degree of freedom; U = upper limit; L = lower limit)

DF	$U_{98\%}$	$L_{98\%}$	$U_{95\%}$	$L_{95\%}$	$U_{90\%}$	$L_{90\%}$
1	6.62	0.00	5.02	0.00	3.84	0.00
2	4.61	0.01	3.69	0.02	2.99	0.05
4	3.33	0.07	2.78	0.12	2.37	0.18
8	2.15	0.21	2.19	0.27	1.94	0.34
16	2.00	0.36	1.80	0.43	1.64	0.49
32	1.67	0.52	1.55	0.57	1.44	0.63
64	1.46	0.64	1.37	0.68	1.31	0.73
128	1.32	0.75	1.26	0.77	1.22	0.79
256	1.21	0.81	1.18	0.83	1.16	0.85
512	1.16	0.86	1.13	0.88	1.11	0.90
1024	1.11	0.89	1.09	0.91	1.07	0.93
2048	1.08	0.91	1.06	0.93	1.05	0.95

measurements, at the same time the amount of information gained by the latter is not greater either. The accurate mapping of the individuals — the sort of sampling analysable from the most sides (SZŐCS, 1977) — is also rather labourconsuming, even with the use of photograph.

The variance analysis is performed with the individual numbers of the successively increased quadrats. In the course of the analysis the quadrat areas duplicate in every case with fusion of the blocks, in such manner that first the quadrats take up oblong shape, then become square again. The individual numbers become summed in the joined quadrats.

The variance between the units of the sample consisting of K quadrats is studies by a nested analysis of variance. The sum of squares belonging to the blocks consisting of r basic unit can be given by the following formula:

$$SSQ_r = \frac{1}{r} \sum_{i=1}^{\frac{K}{r}} X_{i(r)}^2 - \frac{1}{2r} \sum_{i=1}^{\frac{K}{2r}} X_{i(2r)}^2 \qquad (2)$$

$$(K = 2^n \; ; \; r = 2^k \; ; \; k = 1,2,3,\ldots,n)$$

where $x_{i(r)}$ and $x_{i(2r)}$ are the individual numbers measured in the i^{th} block containing r and 2r units, resp. The pertaining mean square, MS_r, is as follows:

$$MS_r = 2r \; SSQ_r/K \qquad (3)$$

Assuming the random distribution of the individuals, each mean square is the estimation of the variance of the Poisson distribution, related to the values measured in the quadrat grid units. In this case the mean square/overall mean is approximately 1 in respect to every block size (PODANI, 1983).

The deviation of the mean square/overall mean value from that of random distribution can be tested by the critical values of the χ^2 probe. The upper (and lower) critical limit can be given by the ratio of the critical χ^2 value belonging to the required error-probability level, as well as by the ratio of the appropriate degree of freedom (THOMPSON, 1958, GREIG-SMITH, 1961):

$$U_{P\%} = \chi^2_{U,P\%}/DF \qquad (4)$$

(The values of the critical limits are summarized in Table 1.).

The following relationships is valid for the confidence limits. If the mean square/overall mean value falls into the critical limits, there is no significant deviation from random distribution. If this value surpasses the upper critical limit, the trend of the deviation is contagious distribution, showing group-formation; while being uniform distribution in case the value is below the lower limit.

The mean square — block size graph usually takes up a serrated curve shape. In case of random distribution the mean square/overall mean value ranges between the two limits, close to 1. If the value shows an elevating peak over the upper limit, this indicates aggregation at the given square size. In such case the $MS/\bar{x}$ increases till the pertaining block size reaches the mean size of the aggregates, groups. If the aggregates themselves also show grouped localization, the $MS/\bar{x}$ remains at almost identical level (PRÉCSÉNYI, 1964, GREIG-SMITH, 1983). In case of aggregates showing random distribution, however, it falls below the critical value again, as also proved by computer-simulations (see Fig. 1).

A computer programme was prepared for performing the analysis, in BASIC programming language for use in Commodore—64 microcomputer. This programme provided to study several properties of the analysis on the computer-generated point-mass of random distribution and on populations of know pattern, and also to compare the distribution of natural populations with "populations" of random distribution.

Orientation of the aggregates

In the course of the analysis the direction of the oblong block can be chosen in two ways. The graphs obtained as a result of the two different analyses will show divergent courses, the peaks will arise at different square sizes (see Fig. 1), except if the distribution is symmetrical to one of the diagonals. Often the size of the peaks (which also indicates the pattern intensity) is not uniform either, which might mean the specific orientation of the aggregates. As also expounded by GREIG-SMITH

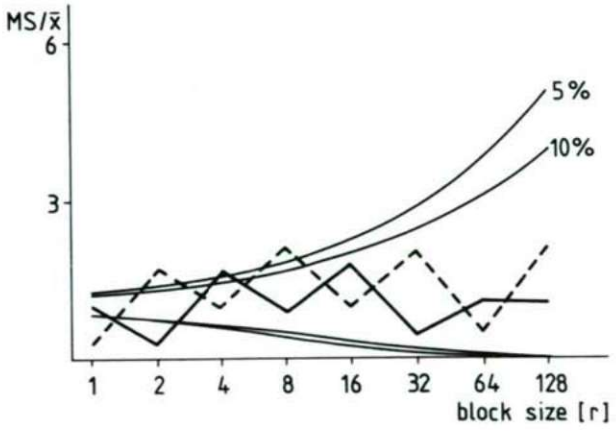

Fig. 1. Graph of $MS/\bar{x}$ against block size for a simulated population, with 95 % and 90 % confidence limits.
Solide line and broken line show different orientations of rectangular blocks during processes (see text).

(1961), in such cases it is more effective to affiliate the units in one direction (along a transection), than to alternating oblongs and quadrats. In this case the peaks are in correlation with the mean linear size of the patches.

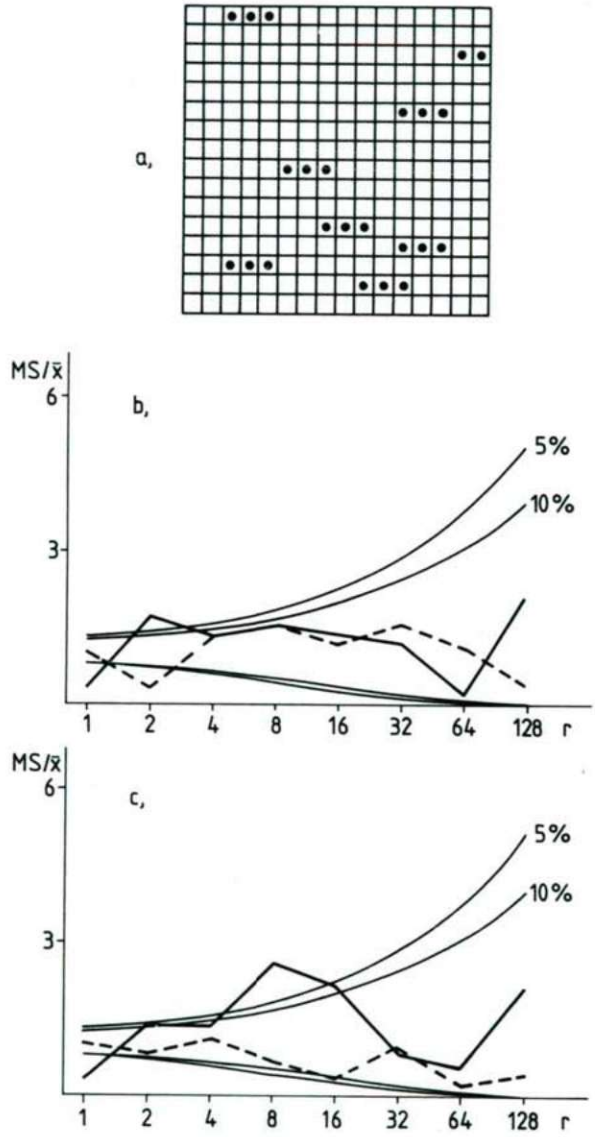

Fig. 2. Graphs of MS/x̄ against block size for simulated population (a). Part (b), shows alternating, while part (c) shows non-alternating grouping Orientation of rectangles is → (solide line) and ↑ (broken line)

The complete fusion of the quadrat grid units, first is one direction then in perpendicular direction, gives the following results. If the aggregates have specific orientation, significant differences will arise between the results of the two analyses

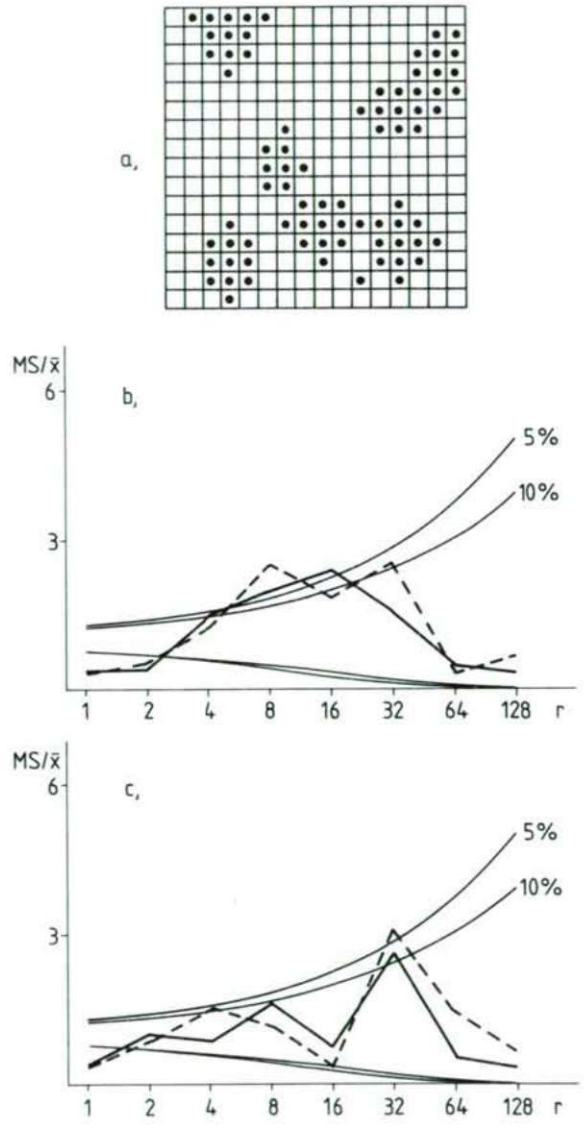

Fig. 3. Graphs of MS/$\bar{x}$ against block size for simulated population (a).
(See labellings for Fig. 2.)

started in different directions (see Fig. 2), while in lack of specific orientation the differences can be neglected. In case the direction of the first fusion corresponds to the orientation of the groups, the peaks may be multiples of the results of the analysis started in perpendicular direction. Fig. 2/a shows such an arrangement where groups of random distribution and identical orientation are present. Fig. 2/b demonstrates the results of the alternate fusion. Only slight differences are between the graphs of the two analyses. A peak indicating aggregation is only observable on one of the curves, while the other runs between the confidence limits.

In case of complete fusion in one direction (Fig. 2/c), two outstanding peaks were the results of the fusion corresponding to the orientation of the aggregates, while again the other curve remained between the confidence limits.

Fig. 3. sets an example for non-elongated patches, where the two parts of both the alternate (Fig. 3/b) and one-way fusion (Fig. 3/c) result close to similar curves.

The centre of the aggregates is of random distribution in both examples. The intensity of the pattern was low in both cases, owing to the low individual numbers.

AN EXAMPLE

In the followings the calculation course of the analysis and the explanation of the results are shown in a concrete example.

The individual numbers set in the basic units of the quadrat grid made up of 16x16(=256) squares seen in Fig. 4. serve as the starting-point of the analysis. The units of the quadrat grid were 10x10 cm in size. (The recording was made in 1984 at Bugacpuszta from the *Achilleo-Festucetum pseudovinae* association. The studied species was the *Plantago maritima)*.

The course of the calculations is observable in Table 2.

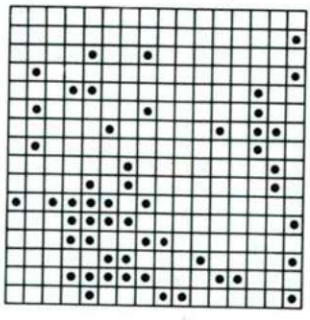

Fig. 4. Map of distribution of *Plantago maritima*

Table 2. Partial results of the calculation

r	S	SSQ	DF	MS	MS/$\bar{x}$	$U_{95\%}$	$U_{90\%}$
1	52.0	20.0	128	0.1563	0.7695	1.26	1.22
2	32.0	8.5	64	0.1328	0.6538	1.37	1.31
4	23.5	3.75	32	0.1172	0.5770	1.55	1.44
8	19.75	2.375	16	0.1484	0.7306	1.80	1.64
16	17.375	3.375	8	0.4219	2.0770	2.19	1.94
32	14.0	0.8438	4	0.2109	1.0383	2.78	2.37
64	13.1563	1.3282	2	0.6641	3.2694	3.69	2.99
128	11.8281	1.2656	1	1.2656	6.2306	5.02	3.84
256	10.5625	—	—	—	—	—	—

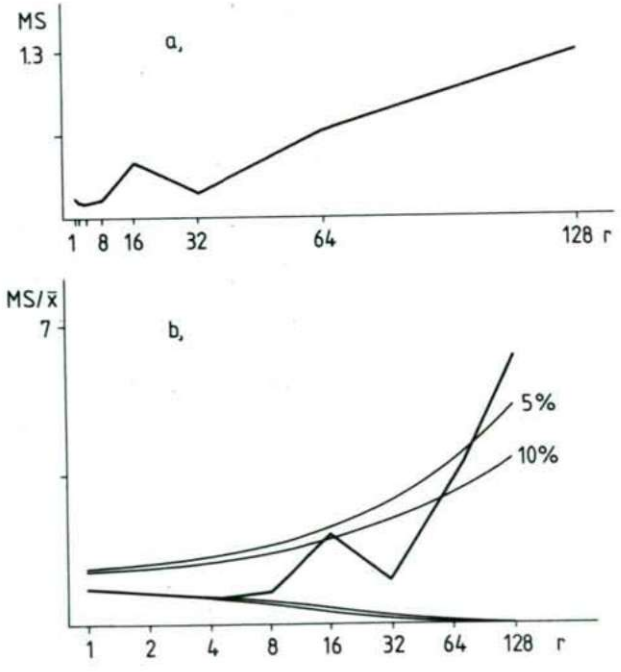

Fig. 5. Graphs of MS against block size (a) and of MS/$\bar{x}$ against block size (b) for *Plantago maritima*

The first column of Table 2 indicates the number of the combined elemental blocks. The second column comprises the series of sums of squares. The differences according to pairs of the sums of squares are observable in the third column (the

second is deducted from the first, the third from the second, etc.). These are actually the values deriving on the basis of the (2) equation.

The values of the fourth column are the degrees of freedom of the successive sums of squares. The value of the degrees of freedom is obtained by taking the difference of the elemental numbers of the consecutive samples. Here, the initial elemental number is 256, and 128 for the sample of 2 block numbers. The difference of the two will be the degree of freedom = 128.

The fifth column shows the ratios of the SSQ values and the degrees of freedom, the variances. The ratio of the variances and the mean individual number is demonstrated in the sixth column. There are 52 individuals in our example, thus the overall mean is 0.203. The last two columns show the upper critical limits of the MS/overall mean, which belong to 5% and 10% error probability level.

The results can be illustrated as the function of the mean square or the mean square/overall mean and the block number, as observable in Fig. 5, with the block size demonstrated on linear or logarithmic scale.

The Figure shows that the variance of the individual numbers increases with the increase in block size. At block size 16 (1600 cm^2) an outstanding peak is observable, then the curve falls back to the following square size.This peak appearing at block size 16 indicates weak aggregation, its deviation from chance distribution is only significant at 10% level.

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References

GREIG-SMITH, P. (1952): The use of random and contiguous quadrats in the study of the structure of plant communities — Ann. Bot. London N.S. *16*, 293-316.

GREIG-SMITH, P. (1961): Data on pattern within plant communities. I. The analysis of pattern — J. Ecol. *49*, 695-702.

GREIG-SMITH, P. (1983): Quantitative plant ecology — Blackwell Sci. Publ. London, 3rd ed.

PODANI, J. (1983): Spatial processes in the analysis of vegetation : theory and review — Acta Bot. Hung. *30*, 75-118.

PRÉCSÉNYI, I. (1964): A note on the problem of homogeneity — Acta Bot. Acad. Sci. Hung. *10*, 217-219.

PRÉCSÉNYI, I., FEKETE, G. and SZUJKÓ-LACZA, J. (1967): Pattern studies in Quercus pubescens wood — Acta Bot. Acta Sci. Hung. *13*, 277-289.

PRÉCSÉNYI, I. (1981): Növénytársulások struktúrája (Structure of plant communities. In: HORTOBÁGYI, T. and SIMON, T. (eds.): Növényföldrajz, társulástan és ökológia. pp. 202-225. — Tankönyvkiadó, Budapest)

SZÖCS, Z. (1977): Újtípusú számítógép-orientált módszerek a vegetáció szerkezetének elemzésére. (New computer oriented methods for the study of vegetation structure) — Vácrátót (Thesis)

THOMPSON, H.R. (1958): The statistical study of plant distribution patterns using a grid of quadrats — Aust. J. Bot. *6*, 322-342.

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CYTOCHEMICAL DETECTION OF NADH-DIAPHORASE POSITIVE NERVE CELLS IN THE ALIMENTARY CANAL OF FROG

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Abstract

Authors studied the nerve cells of the myenteric plexus of the frog alimentary canal using the NBT/NADH-diaphorase technique, as well as silver impregnation and transmission electron microscope. It was concluded that the nerve cells form groups of ganglia in the stomach; on the contrary they show dispersed localization in the small intestine, while both ganglionic and solitary neuronal arrangements are detectable in the transitional duodenal section. The size of the nerve cells varies through the length of the intestinal canal (50—200 μm^2). The myenteric plexus is situated in the connective tissue of the subserosa, in this way differing from the higher Vertebrates plexuses. The neuronal density ranges from 600 to 1100 cells/cm^2 throughout the lenght of the alimentary canal. The organization of the cellular elements shows a transition between the innervation by dispersed nerve cells and the enteric nervous system of ganglionic arrangement.

Key words: frog intestine, myenteric plexus, NBT/NADH diaphorase, "whole mount", cell count

Introduction

The structural organization of the myenteric plexus has been studied by several authors at various sections of the alimentary canal (FILOGAMO and VIGULIANI, 1954; GABELLA, 1971; IRWIN, 1931; LEAMING and CAUNA, 1961; OKHUBO, 1936a and b). The studies firstly deal with the innervation of the mammal intestine, and in this relation also give account on cell count data referring to area unit. Studies of this kind have been launched by the NADH-tetrasolium reductase technique, introduced by GABELLA, (1969). This technique electively stains the nerve cells with good reproducibility, and contrary to the classical staining procedures, demonstrates practically every nerve cell. In the possession of this specific and highly effective technique, it seemed obvious to study the myenteric plexus of the frog (*Rana esculenta* L.) enteric nervous system, since literary data are rather scant in respect to its organization — particularly in regard to the upper sections of the intestinal canal (GUNN, 1951). The aim of our studies was to examine the myenteric plexus of the frog intestine from morphological aspect, and within this, by means of the detection

and counting of the NADH-diaphorase positive nerve cells, we wished to obtain new (comparative) data on the innervation of the studied intestine sections.

Materials and methods

Mature male and female adult frogs (*Rana esculenta* L.) were used in our studies. The method of GABELLA (1969) was used for the histochemical reaction. The animals were decapitated and the distal end of the intestinal canal was tied down, then the intestine was filled up with Krebs-solution. After filling up, the other end of the intestine was also tied down, then the whole intestinal tract was placed into 0.3% Triton-solution, followed by washing for 2x5 minutes. Then the filled up intestine was placed into incubating solution, the composition of which was the following: 2.5 mg of NBT dissolved in 5 ml of tridistilled water, 5 ml of 0.1 M phosphate buffer (pH 7.3), 10 mg of NADH dissolved in 10 ml of tridistilled water. The intestine was kept in the incubating solution till the appearance of the dark blue colour reaction (40—60 min). The fixation of the samples in 10% neutral formaline lasted for at least 12 hours. Using safety razor blade, about 1 cm slices were cut from the intestine, perpendicularly to the longitudinal axis, and "whole mount" specimens were prepared from the slices. During the preparation of the specimens it turned out that the complete muscle layer (tunica muscularis) could be removed without damage to the nerve plexus.

The method of BIELSCHOWSKY-GROS-CAUNA was used for the silver impregnation (LEAMING and CAUNA, 1961).

For the purpose of electronmicroscopic studies, transcardial perfusion was performed with fixative containing 1% glutaraldehyde and 2% paraformaldehyde, 0.2 M Na-cacodylate buffer was used for the preparation of the fixative. Following 2 hours of immersion post-fixation, the material was washed in 0.2 M phosphate buffer containing, 7.5% saccharose. 2% buffered osmium was used for osmification, then the material was dehydrated in ascending alcohol series. During dehydration "block contrasting" was performed in the solution of 75% alcohol saturated with uranylacetate. Using propylene-oxide intermedium, the samples were embedded in Durcupan. The ultrathin sections were also contrasted with lead citrate according to REYNOLDS. The electronmicroscopic figure was prepared by Tesla BS 540 electronmicroscope.

Results

The light microscopic studies of the specimens prepared by the NADH-diaphorase cytochemical reaction showed the morphology of the myenteric plexus to be strongly variable throughout the length of the intestinal canal. Using the present method to study the esophagus could not be accomplished since the proximal end of the intestine could only be tied down on the account of this section of the intestine.

The nerve cells form groups of ganglia at the area of the stomach (Fig. 1), with 3—10 neurons occupied in each ganglion. The average distance between the ganglia is about 100 μm, but distances of only 30—40 μm were also found between some neighbouring ganglia. The ganglia of the stomach are generally polygonal. The section of the duodenum close to the stomach assumes an interesting appearance from the viewpoint of neuronal arrangement. Solitary neurons are frequent here, with the simultaneous occurrence of ganglional arrangement (Fig. 2). The size of the cells shows great variation. The cell profile ranges from 50 to 200 μm^2 in case of both

the stomach and the proximal end of the duodenum. In the stomach, about 50% of the cells are large: more than 150 μm^2, one third are small (50—75 μm^2), while the remaining parts show moderate profile. From the processes of the nerve cells, generally only one can be followed till a greater distance from the cell body (Fig. 3a). The histochemical reaction causes well detectable granulation in the plasma of the nerve cells, and it can be observed well close to the cell body, in the initials of the processes (Fig. 3b).

The ganglionic arrangement of the nerve cells disappears completely at the distal part of the duodenum as well as in the small intestine: the cells are arranged sporadically without showing even distribution (Fig. 4). Here the localization of neurons from each other is at distances of 25—75 μm.

In the environs of the blood vessels entering from the mesenterium, the neuronal density of the gut is higher then on the antimesenterical side. The profile of the neurons is small (50—75 μm^2). A few larger neurons are also occassionally observable at the distal end of the duodenum and in the small intestine, but these are consequently found among the entering of mesenterial vessels (Fig. 5a). The size of these neurons surpasses that of the large neurons of the 200—250 μm^2 profile. These large neurons are multipolar (Fig. 5b). The neurons were found in the sections of duodenum and small intestine, resp., had one or two processes (Fig. 6).

The neurons were counted per intestinal section, and the results obtained are given in Table 1. The number of neurons per area unit differs according to various intestinal sections. The lowest amount was found at the initial sections of the stomach (619 cells/cm^2), while an increase was manifested at the pylorus section (948 cells/cm^2).

The duodenum cell count showed slight decrease compared to the pylorus (772 cells/cm^2), but the rest of the small intestine parts was found to be more abundant in nerve cells: the number of cells was found to be the highest here from the studied sections (about 1000 cells/cm^2). At the same time, no significant difference was manifested in respect to cell number between the proximal and distal ends of the small intestine, as observable in the column diagram (Fig. 7).

The results of the NADH-diaphorase procedure serving for the detection of the neurons evidence the majority of the neurons are localized on the outer surface of the tunica muscularis. This arrangement has also been verified by the results of the silver impregnation and electron microscopic studies. The silver impregnation light microscope figure (Fig. 8b) shows that the myenteric plexus is situated on the outer surface of the muscle layer, and this is supported by the figure prepared from the specimen labelled by the diaphorase technique (Fig. 8a). On the electron microscopic picture (Fig. 9) this arrangement is even clearer: the edge of the smooth muscle layer; the centrally located neuron and the neuropile in the direct neighbourhood; furthermore, the wide connective tissue layer of the consecutive subserosa, mainly composed of collagen fibers are all well recognizable in the left upper corner of the Figure.

Discussion

It is evident from both literary data (GABELLA, 1969; 1971; 1979) and our study results that the histochemical detection of NADH-diaphorase activity is suitable for the selective demonstration of the nerve cells, for the determination of neuron number, as well as for studies on the cellular arrangement in the frog intestinal canal.

The localization of the myenteric plexus is somewhat different from that is usual in the case of mammal species. The cellular elements are situated on the outer side of the tunica muscularis, while in the case of higher Vertebrates the usual localization is between the circular and longitudinal layers of the muscle sheat. The cause of the deviation might be the weak development of the longitudinal muscle layer, since this layer stands of only 1—4 cell rows in frogs, thus the axons necessary for innervation can easily penetrate through it. Our observations regarding the shape of the diaphorase-positive nerve cells is in correlation with the data of GUNN, (1951) obtained by the silver impregnation method, according to which both small and large cells are found in the small intestine; furthermore, the localization of the large cells rarely detectable at this site is also thought by this author to be in the surroundings of the large veins entering from the mesenterium. There have been no data so far concerning to the arrangement of the nerve cells in the frog stomach. Our studies have proved on the one hand the ganglional arrangement, on the other hand that the small and large neurons in the stomach ganglia are found simultaneously beside each other.

DOGIEL (1896, 1899) classified the vegetative neurons on the basis of their processes. Nowadays, however, it seems more expedient to take the cell size into consideration too, according to the suggestion of LEAMING and CAUNA, (1961) as well as GUNN, (1959, 1968). Based on newer studies, a third basic cell type can also be found in the myenteric plexus of the chicken intestinal tract (CSOKNYA and BENEDECZKY, under publication).

Gunn also had made an attempt to differentiate the small and large neurons from the viewpoint of transmitter content, however, she could not achieve unambiguous results (GUNN, 1968). Newer studies (VINCENT et al. 1983a and b, 1986) have shown that the comparison of the results of the diaphorase reaction with those of the acetylcholine-esterase reaction can successfully be applied in the central nervous system. Performing the reactions separately, the neurons giving both histochemical reactions could be identified on consecutive sections. Supporting the result of the acetylcholine-esterase reaction with choline-acetyltransferase reaction, VINCENT et al. (1983a) came to the conclusion that the NADPH-diaphorase positivity of certain central nervous system structures assumes cholinergic transmission. In their other studies, however, these authors could not unambiguously support this statement (VINCENT et al. 1983b). In a recent publication, VINCENT et al. (1986) have been successful in proving the coexistence of the cholinergic-peptidergic system in respect to the ascending reticular cholinergic systems. In our opinion, both the cholinergic and adrenergic neurons of the intestinal myenteric plexus became

stained during the course of our experiments. This statement is supported by the fact, that in case of consecutive diaphorase- and acetylcholinesterase reaction performed on the same "whole mount" preparation, such cells are found that show only diaphorase-activity (GÁBRIEL, unpublished data).

We could not find any literary data on nerve cell count concerning the frog intestine. Our data show 2—7 times less cells per intestinal section than the summarizing data of GABELLA, (1979) related to mammal species. There are no data, however, on the tissue volume innervated by certain ganglia or cells. Calculations in this respect are rather difficult since the muscle layer is not of the same thickness even throughout the same intestinal section. On the other hand, distending with Krebs-solution produces the elongation of the intestinal muscle cells. Therefore, these do not show their original volume during the course of measurement, and preliminary measurements are not possible owing to the filling up the whole intestine.

Our present results suggest that the development of the myenteric plexus of the frog intestine shows an intermediary state phylogenically at the various sections of the intestine: the myenteric plexus of the stomach is ganglionic, the small intestine contains sporadically arranged nerve cells. In case of chicken as well as mammals, it is known (DOGIEL, 1896, 1899) that the whole myenteric plexus shows ganglional arrangement. The alimentary canal of the developing chicken and mammal, however, does not show clearly this kind of arrangement, since the cells still migrate and there is also an increase in their absolute number with further development (GABELLA, 1971).

Our results obtained with silver impregnation and transmission electron microscope have unambiguously supported our assumptions regarding the localization of the plexus, according to which the nerve plexus is not localized at the "usual" place between the two muscle layers, but rather outside this, in the subserosa.

The morphological and functional significance of this characteristic localization is to be clarified by further studies.

References

CSOKNYA, M. and BENEDECZKY, I. (1986): Cell types of the enteric nerve plexuses in the chicken. — Acta Biol. Szeged. *32*, 93-102.

DOGIEL, A.S. (1896): Zwei Arten sympatischer Nervenzellen. — Anat. Anz. *11*, 679-687.

DOGIEL, A.S. (1899): Über den Bau der Ganglien in den Geflechten des Darmes und der Gallenblase des Menschen und der Saugetiere. — Arch. Anat. Physiol. *5*, 130-159.

FILOGAMO, G. and VIGULIANI, F. (1954): Richerche sperimentali sulla correlazione tra estenzione del territorio di innervazione e grandezza e numero delle cellule gangliari del plesso mienterico (di Auerbach) nel cane. — Riv. patol. nerv. ment. *75*, 1-32.

GABELLA, G. (1967): Neuron number in the myenteric plexus in newborn and adult rats. — Experientia *23*, 52-54.

GABELLA, G. (1969): Detection of nerve cells by a histochemical technique. — Experientia *25*, 218-219.

GABELLA, G. (1971): Neuron size and number in the myenteric plexus of the newborn and adult rat. — J. Anat. *108*, 81-95.

GABELLA, G. (1979): Innervation of the gastrointestinal tract. — Int. Rev. Cyt. *59*, 129-193.

GUNN, M. (1951): A study of the enteric plexuses in some amphibians. — Q. Jl. Microsc. Sci. *92*, 55-77.
GUNN, M. (1959): Cell types in the myenteric plexus of the cat. — J. Comp. Neurol. *111*, 83-100.
GUNN, M. (1968): Histological and histochemical observations on the myenteric and submucous plexuses of mammals. — J. Anat. *102*, 223-229.
IRWIN, D.A. (1931): The anatomy of Auerbach's plexus. — Am. J. Anat. *49*, 141-166.
LEAMING, D.B. and CAUNA, N. (1961): A qualitative and quantitative study of the myenteric plexus in the small intestine of the cat. — J. Anat. *95*, 160-169.
OKHUBO, K. (1936a): Studien über das intramurale Nervensystem des Verdauungskanals II. Die plexus myentericus und plexus subserosus des Meerschweines. — Jap. J. Med. Sci. Anat. *6*, 31-37.
OKHUBO, K. (1936b): Studien über das intramurale Nervensystem des Verdauungskanals III. Affe und Mensch. — Jap. J. Med. Sci. Anat. *6*, 219-247.
VINCENT, S.R., SATOH, K., ARMSTRONG, M.D. and FIBIGER, H.C. (1983a): NADH-diaphorase: a selective histochemical marker for the cholinergic neurons of the pontine reticular formation. — Neurosci. Lett. *43*, 31-36.
VINCENT, S.R., STAINES, W.A. and FIBIGER, H.C. (1983b): Histochemical demonstration of separate populations of somatostatin and cholinergic neurons in the cat striatum. — Neurosci. Lett. *35*, 111-114.
VINCENT, S.R., SATOH, K., ARMSTRONG, D.M., PANULA, P., VALE, W. and FIBIGER, H.C. (1986): Neuropeptides and NADPH-diaphorase activity in the ascending cholinergic reticular system in the rat. — Neuroscience *17*, 167-182.
WALKER, D.G. (1963): A survey of dehydrogenases in the various epithelial cells in the rat. — J. Cell. Biol. *17*, 255-277.

Table 1. Results of the cell count

Section of the intestine	cell count (total)	area (mm^2)	cells/unit ($nu./cm^2$)	average
stomach cardia	524[a]	72	728	619
	523[b]	104	503	
	634[c]	101	627	
stomach pylorus	241[a]	21	1100	948
	445[b]	48	927	
	393[c]	48	818	
duodenum	363[a]	43	844	772
	462[b]	66	700	
small intestine proximal	367[a]	45	814	966
	379[b]	35	1083	
	210[c]	21	1000	
small intestine distal	351[a]	36	975	1004
	124[b]	12	1033	

The labellings a, b, c mean the cell count data of the total amount of specimens prepared from three different experimental animals.

Legend to figures

Fig. 1. The majority of the nerve cells in the stomach form groups of ganglia (arrow.) 200 x

Fig. 2. Both ganglional and solitary neurons are observable in the transitional section of the stomach-duodenum. 200 x
G: ganglion, S: solitary cell

Fig. 3a. In general, the cells possess well detectable processes. 400 x

Fig. 3b. Strong granulation can mainly be observed in the neuronal processes (arrow.) 800 x

Fig. 4. The neurons of the small intestine do not form groups of ganglia and do not show even distribution (arrows: cells). 200 x

Fig. 5a. Large neurons can be seen (arrows) in the surroundings of the vessels entering from the mesenterium. 200 x

Fig. 5b. Several processes are recognizable (arrows) projecting from the large cells. 800 x

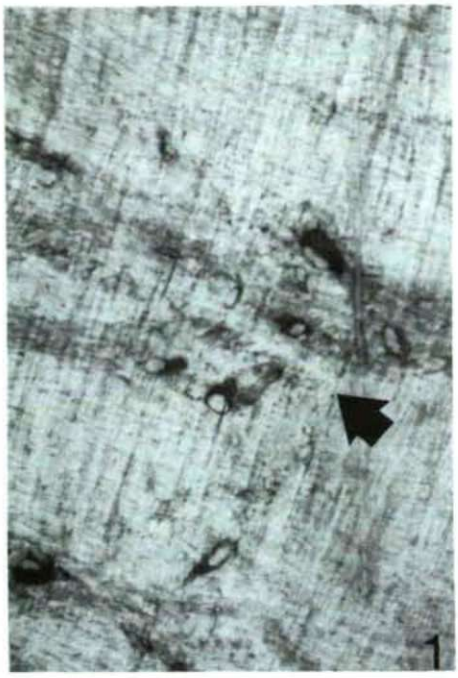
Fig. 6. The small neurons mostly possess 1 — 2 processes at the area of the small intestine. The processes are indicated by arrows. 200 x

Fig. 7. On the column diagram the neuron density of the intestinal canal can be seen according to the various sections of the intestine.

Fig. 8a. The plexus is localized outside the muscle layer: smc: smooth muscle cells, n: neuron. 200 x

Fig. 8b. The plexus (indicated by arrows) is situated at the outer edge of the muscle layer, beneath the connective tissue limiting the intestine from outside. 200 x

Fig. 9. Detail of the myenteric plexus. In the upper left corner of the Figure the detail of a smooth muscle cell (smc) is observable, with a neuron (n) in the neighbourhood, close to which a neuropil (np) can be seen. The thick layer of the subserosa, packed with the collagen fibres (co), is situated near the outer surface of the intestine. 25 000 x



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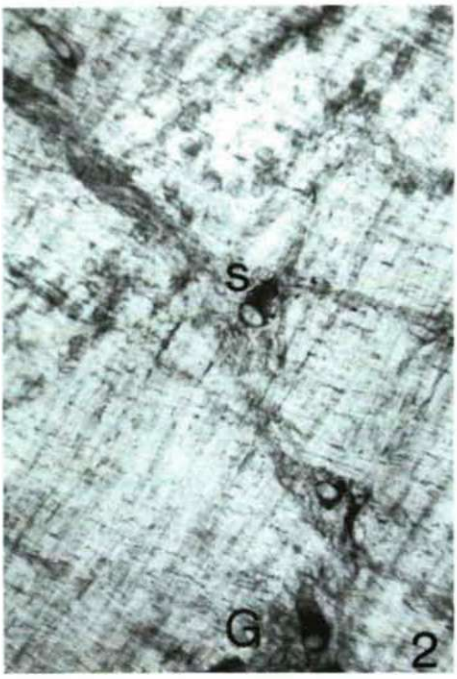

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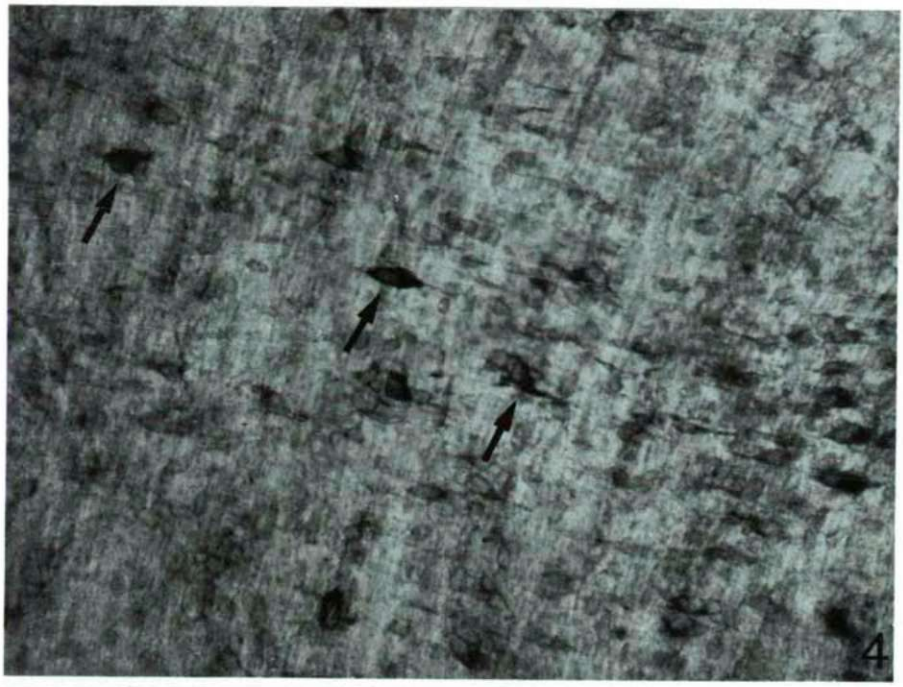
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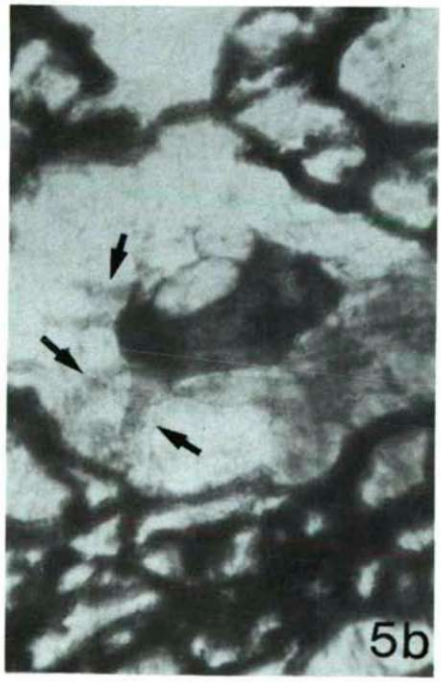
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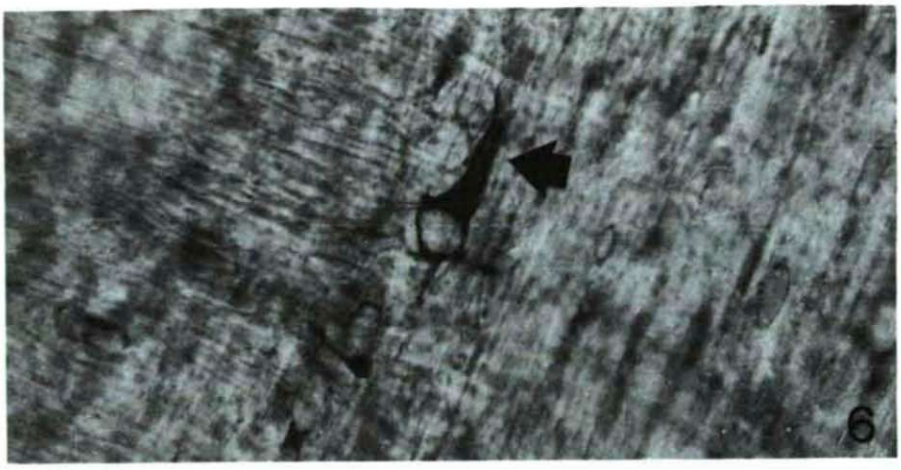
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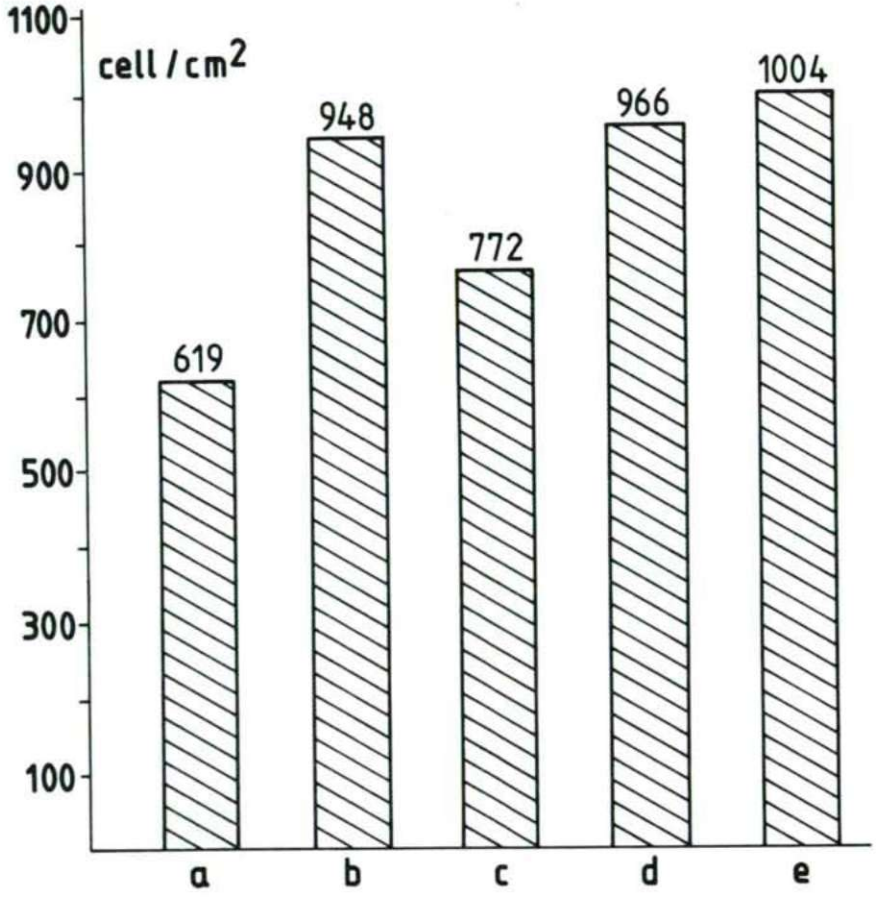
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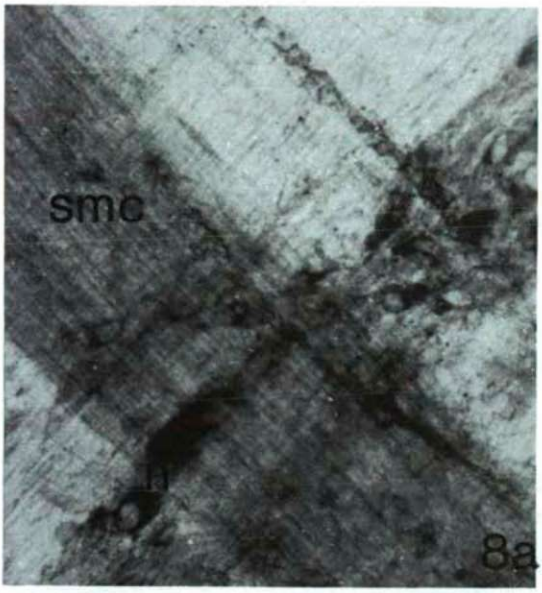
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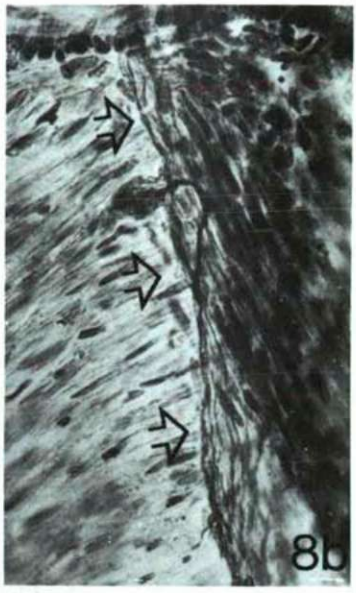
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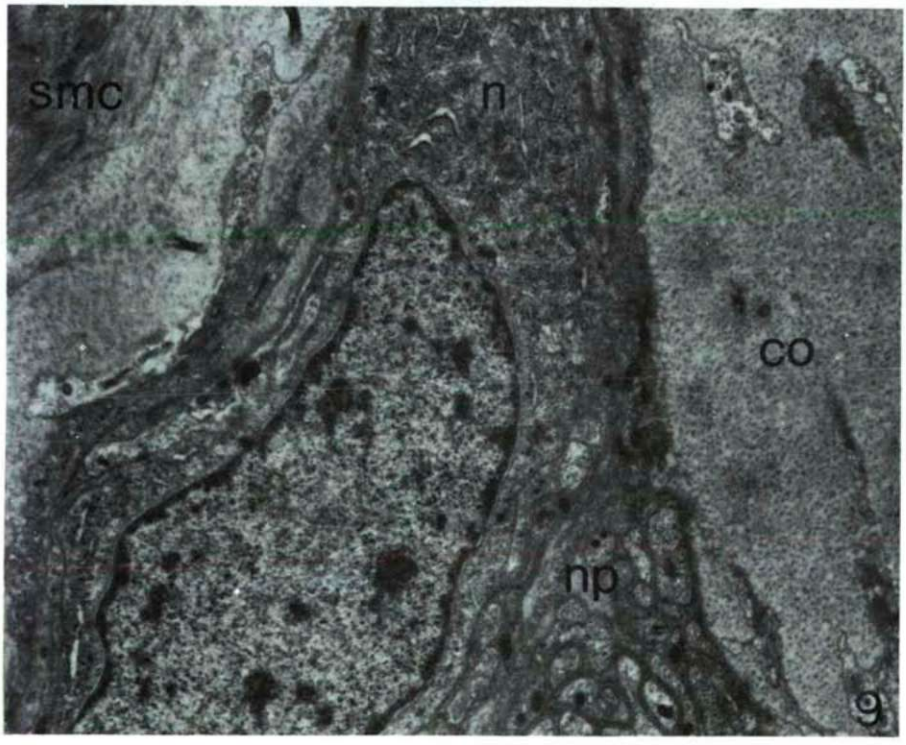
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ACTA BIOL. SZEGED. 33. PP. 97—104 (1987)

FLUORESCENCE CHARACTERIZATION OF THE NERVE PLEXUSES IN THE SMALL INTESTINE OF THE CHICKEN

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Abstract

The enteric nerve plexuses in the small intestine of the chicken has been investigated in sections and stretch preparations by means of the silver staining and the glyoxylic acid histofluorescence methods. Both techniques revealed the presence of two submucosal plexuses in the small intestine of the chicken. Using the histofluorescence technique some of the nerve cell bodies located in the perivascular plexuses showed monoamine-specific fluorescence. The appearance and distribution of nerve elements in the chicken small intestine has been studied also with electronmicroscope. Profiles containing dense-core vesicles in different sizes have been claimed to contain catecholamines.
Key words: enteric nerve plexuses, catecholamine, chicken, small intestine

Introduction

Most of the structural and functional studies concerning the organization of the enteric nervous system were performed on mammalian intestine (GUNN, 1968; GABELLA, 1979; FURNESS and COSTA, 1980; SCHEUERMAN and STACH, 1984).

Data on the innervation of the alimentary tract of the birds were mostly based on staining techniques as methylene blue and silver methods (ÁBRAHÁM, 1936; CSOKNYA and BENEDECZKY in print; KOLOSSOW et al. 1932; MICHAIL and KARAMANDLIDIS, 1967). Using fluorescence histochemical methods (BENNETT and MALMFORS, 1970) it was shown that the basic pattern of the adrenergic innervation of the alimentary tract in the birds were comparable to that in mammals (COSTA and GABELLA, 1971; STACH, 1984). All these techniques revealed several contrary concerning the organization of the enteric nervous system. Because of the very small number or even the lack of fluorescent nerve cell bodies observed in the alimentary tract of the chicken (READ and BURNSTOCK, 1968; BENNETT and MALMFORS, 1970; ALI and MCLELLAND, 1978) it has been suggested that all the catecholamine (CA) containing axons in the intestine of the domestic fowl arise from extrinsic ganglions. Some authors report on a secondary meshwork of submucosal plexus (ALI and MCLELLAND, 1978). On the bases of ultrastructural studies (BENNETT and COBBS, 1969) the majority of the terminals contained a mixed

population of small and large granular vesicles which suggest that at least some of the nerves handle with catecholamines.

During present work using silver impregnation and glyoxylic acid induced fluorescence methods we tried to clear up some of these contraversaries.

Materials and methods

The small intestine of the 6 days old chicken was investigated. The basic histological structure of the small intestine was studied by hematein-eosin stained paraffin sections. Detection of the enteric nerve plexuses was carried out on sections (30 μm) stained by BIELSCHOWSKY-GROS-CAJAL silver method.

For the histochemical detection of monoamines the sucrose-phosphate-glyoxylic acid (SPG) method (DE LA TORRE and SURGEON, 1976) was applied to wholemount stretch preparates of chicken small intestine. After dissection, the mucosal and muscle layers were separated and incubated in reaction mixture containing 6.8 g sucrose, 3.2 g KH_2PO_4 and 1 g glyoxylic acid (GA) in 100 ml of distilled water at 4°C for 25 min. The layers were then stretched separately on microscope slides, blotted with blotting paper and dried under cool air for about half an hours. Finally the specimens were placed in an oven at 95°C for 4 min, and mounted with liquid paraffin. The preparations were viewed through a Leitz Orthoplan microscope equipped with HBO 50 W super pressure mercury lamp and an E-3 filter block. Tissue blocks for electron microscopy were taken after vascular perfusion with ice-cold KARNOWSKY fixative. Small pieces of the small intestine were kept in the same fixative for 2 hours. After postfixation in 2% osmium teroxid the specimens were dehydrated in ascending ethanol series and embedded in Durcupan. Tissue blocks were contrasted with saturated uranyl acetate in 75% ethanol and sections were recontrasted with lead citrate, then studied under TESLA BS 500 electronmicroscope.

Results and discussion

The basic histological structure of the chicken small intestine is similar to that of mammalian pattern (Table 1.a.). The thick epithelium is characteristic to the absorptive tissues. The muscularis mucosae consist of one longitudinal layer of smooth muscle cells. The submucosa is reduced to a few connective tissue fibres. The muscularis externa has a highly developed inner circular layer and a relatively poorly developed outer longitudinal layer covered by the tunica serosa in the whole length of the small intstine. Both the histochemical and histological methods we

Table 1.

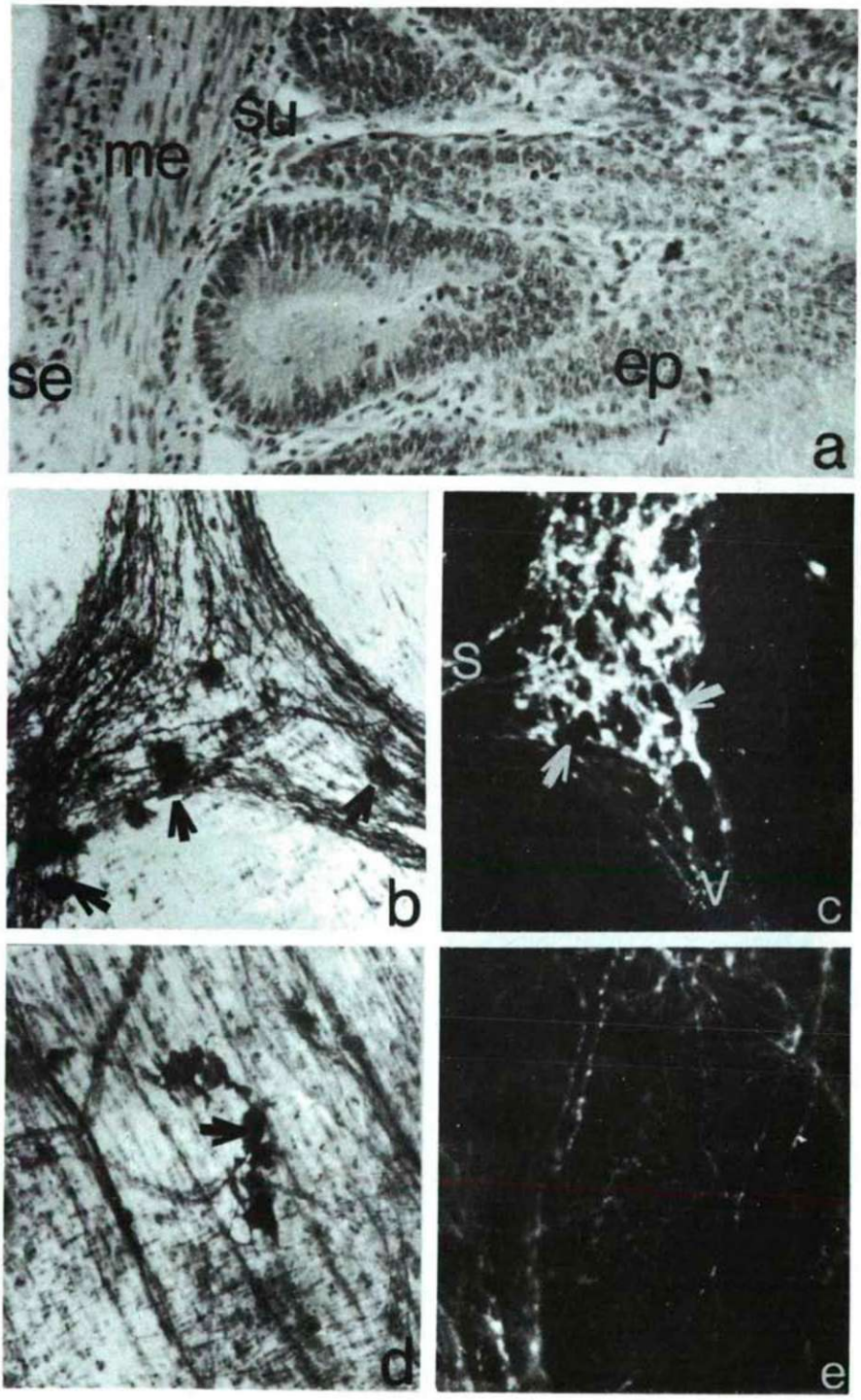
a. Detail of a hematein-eosin stained cross section from the small intestine of the chicken. ep—epithelium, su—submucosa, me—muscularis externa, se—serosa x 450

b. One ganglion in the plexus myentericus after silver impregnation. Arrows indicate the large, mainly multipolar nerve cells. x600

c. Ganglion in the plexus myentericus after GA induced fluorescence. Dense holes (arrows) represent the non-fluorescence cell bodies in the ganglion. s—smooth fibres, V—varicose fibres x600

d. Silver impregnated fibres of the plexus submucosus externus, characterized by ganglions with a few cell bodies (arrows) and a small number of fibres. x 600

e. Fine network of fibers from plexus submucosus externus, with a very intense GA induced fluorescence. x 600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used during the present studies were suitable to visualize the enteric nerve plexuses in the small intestine of the chicken (Table 1.b-e., 2.a-e.). After silver impregnation the nerve fibers of the plexus myentericus appeared as a compact aggregates between the longitudinal and circular muscle layer (Table 1.b.). All the nerve cell bodies were located in ganglions (Table 1.b.c.). Both in the silver impregnated sections and on the GA treated wholemounts two networks of plexus submucosus were identified (Table 1.d.e., 2.a.b.). In the plexus submucosus internus (Table 2.b.) which lies close to the tunica muscularis mucosae the individual nerves could much better be recognized than in plexus myentericus. This plexus forms a regular pattern with fibers running to the mucosa. The plexus submucosus externus can be recognized as a separate entity lying close against the inner side of the circular muscle layer. Most of the nerve cell bodies are also aggregated into ganglions although the sizes of the cells and the ganglions are smaller than in Auerbach's plexus. It was demonstrated with glyoxylic acid that a large part of these nerves were catecholaminergic. The myenteric plexus contains the greatest density of the catecholaminergic fibers (Table 1.c.). At many places in the ganglions large dense holes surrounded by basket-like fluorescent varicose and non-varicose axons have been observed (Table 1.c.). It is highly probable that dense holes related to nonfluorescent enteric nerve cell bodies. The smooth appearance of some nerves and varicosity of others might be the indication of the different role of these nerves. We suppose that the varicose segments represent the active segments in chemical neurotransmission while the smooth have mainly conducting role. The submucosal plexus in stretch preparations were distributed in two discrete plane (Table 2.b.). In plexus submucosus internus almost all the fibers were varicose in appearance (Table 2.b.) The fibers of the plexus submucosus externus have a less intense fluorescence (Table 1.e., 2.b.) which suggests a lower concentration of catecholamine. The fibers of the plexus submucosus internus were always in close connection to the perivascular plexuses (Table 2.d.).

The topographical difference between the two submucosal plexuses might suggest functional difference, too. Our suggestion is that the fibers of the primary or external meshwork may influence mucosal function directly however the fibers of

Table 2.

a. Plexus submucosus internus after silver impregnation. Single nerve cell body is characteristic to this plexus. x 600

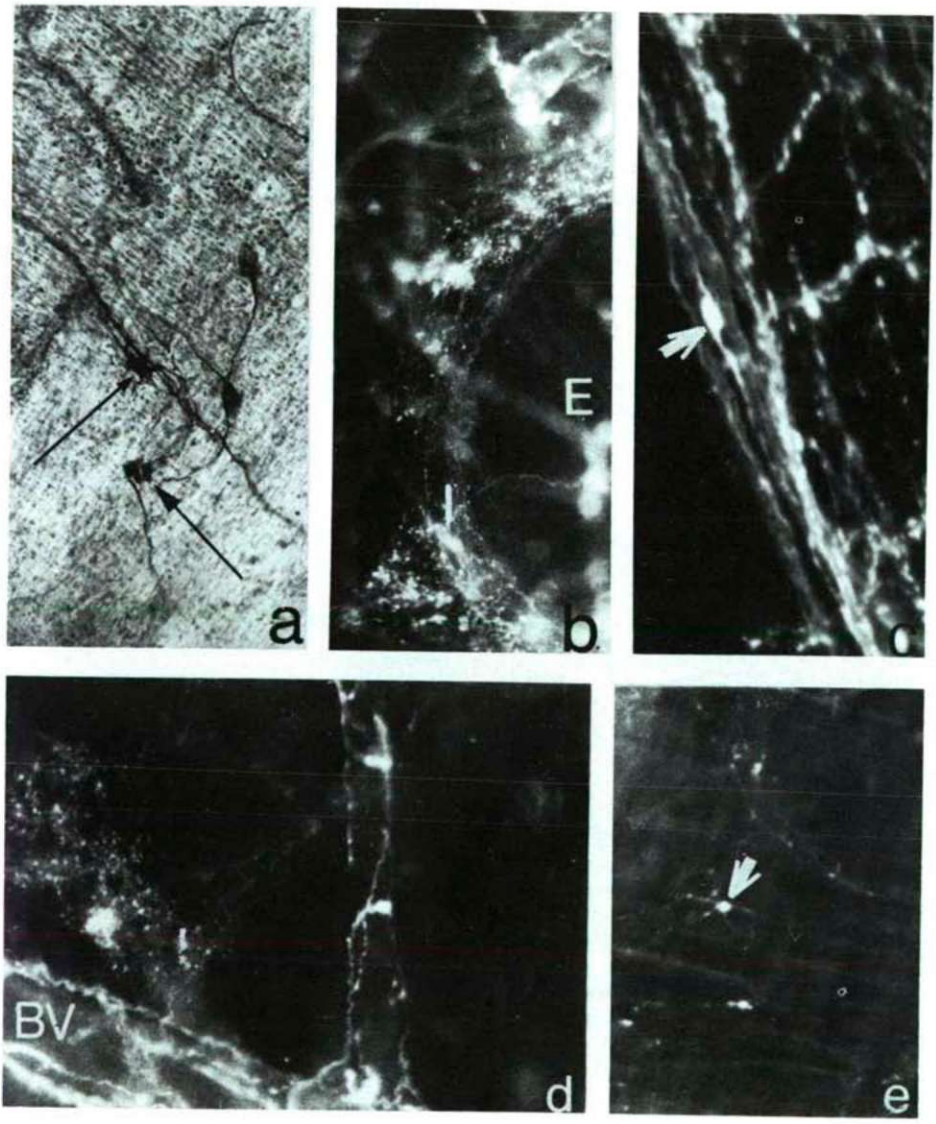
b. GA induced picture of plexus submucosus internus (I) superlayered on the plexus submucosus externus (E). x 600

c. GA induced nerve cell body (arrow) in the perivascular plexus of the chicken small intestine. x 600

d. Close relation of the plexus submucosus internus (I) and blood vessel (BV) in the chicken small intestine. Both the submucosus and the perivascular plexuses show and intensive GA induced fluorescence. x 600

e. Small soliter multipolar cell (arrow) in the intestinal wall, with a well defined GA induced fluorescence. x 800

the inner plexus act by influencing the blood flow. Even though there are a lot of contrary about the ultrastructural appearance of aminergic nerves in the gut, profiles containing dense-core vesicles in different size have been claimed to contain catecholamines (BENNETT and MALMFORS, 1970; GORDON-WEEKS, 1981). Accepting this agreement our present ultrastructural observations indicate the presence of catecholaminergic nerves in both the myenteric and submucosus

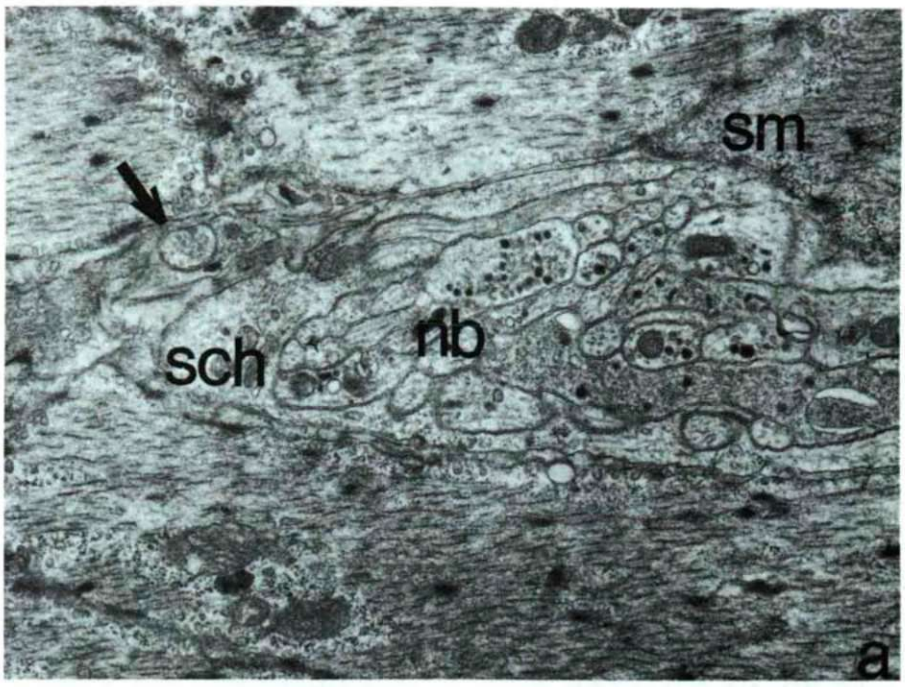


plexuses of the chicken small intestine. In a good agreement with our light microscopic investigations the number of nerve bundles was much higher in myenteric plexus than in submucous plexus. Most of the axons were grouped into bundles (Table 3.a.) lying mainly parallel to the smooth muscle cells. Profiles in nerve bundles were sometimes surrounded by Schwann-cell processes while others were in close association with the muscle cells (Table 3.a.) The axon profiles in both plexuses contained heterogeneous vesicle population even though the dominancy of dense-core vesicles was characteristic. The diameter of these vesicles was around 50—100 Å (Table 3.a.b.). This indicates that most of the nerves in the enteric plexuses handle different kind of catecholamines. Besides transmitter vesicles the axon profiles contain mitochondria and multivesicular bodies. These detailed results allow us to make the conclusion that large part of the nerves in the enteric plexuses in the chicken small intestine are catecholaminergic, and except the perivascular plexuses, where intensively fluorescent cell bodies were noticed all the aminergic nerves are extrinsic in origin.

Table 3.

a. Nerve bundle in the chicken small intestine (nb), surrounded by glial processes (sch). Arrow shows a neuromuscular junction. sm—smooth muscle cell. x 13 000

b. Nerve bundles with different axon profiles in the chicken small intestine. Arrow shows a characteristic profile with heterogeneous vesicle population: dense-core (50—100Å in diameter), small agranular and flattened vesicles can be seen. x 22 000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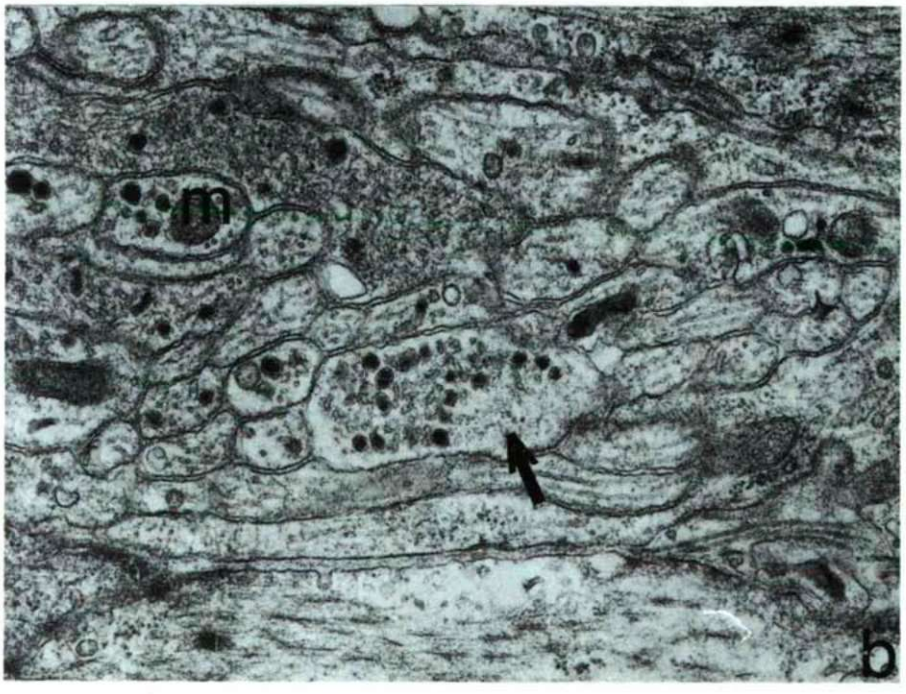
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References

ALI, H.A. and MCLELLAND, J. (1978): Avian enteric nerve plexuses. A histochemical study. — Cell Tissue Res. *189*, 537-548.

ÁBRAHÁM, A. (1936): Beitrage zur Kenntnis der Innervation des Vogeldarmes. — Z. Zellforsch. *23*, 737-745.

BENNETT, T. and COBB, J.L.S. (1969): Studies on the avian gizzard: Auerbach's plexus. — Z. Zellforsch. *99*, 109-120.

BENNETT, T. and MALMFORS, T. (1971): Fluorescence histochemical observation on Auerbach's plexus and the problem on the inhibitory of the gut. — J. Physiol. *218*, 77-78.

COSTA, M. and GABELLA, G. (1971): Adrenergic innervation of the alimentary canal. — Z. Zellforsch. *122*, 357-377.

CSOKNYA, M. and BENEDECZKY, I. (1986): Cell types of the enteric nerve plexuses of the chicken (*Gallus domesticus* L.). — Acta Biol. Szeged, *32*, 93-102.

FURNESS, J.B. and COSTA, M. (1980): Types of nerves in the enteric nervous system. — Neurosci. *5*, 1-20.

GABELLA, G. (1979): Innervation of the gastrointestinal tract. — Int. rev. Cytol. *59*, 129-193.

GORDON-WEEKS, P.R. (1981): Neuroscience properties of nerve endings with small granular vesicles in the distal colon and rectum of the guinea-pig. — Neurosci. *6*, 1-20.

GUNN, M. (1968): Histological and histochemical observations on the myenteric and submucous plexuses of mammals. — J. Anat. (Lond.) *102*, 223-239.

KOLOSSOW, N.G., SABUSSOW, G.H. and IWANOW, J.F. (1932): Zur Innervation des Verdauungskanales der Vögel. — Z. mikr. anat. Forsch. *30*, 257-294.

MICHAIL, S. and KARAMANDLIDIS, A. (1967): Morphologie du plexus myentérique d'Auerbach de l'intestine. — Acta anat. *67*, 424-436.

READ, J.B. and BURNSTOCK, G. (1968): Comparative histochemical studies of adrenergic nerves in the enteric plexuses of vertebrate large intestine. — Comp. Biochem. Physiol. *27*, 505-517.

SCHEUERMANN, D.W. and STACH, W. (1984): Fluorescence microscopic study of the architecture and structure of an adrenergic network in the plexus myentericus (AUERBACH), plexus submucous externus (SCHABADASCH) and plexus submucous internus (MEISSNER) of the porcin small intestine. — Acta anat. *119*, 49-59.

DE LA TORRE, J.C. and SURGEON, J.W. (1976): Histochemical fluorescence of tissue and brain monoamines: results in using the sucrose-phosphate-glyoxylic acid (SPG) method. — Neuroscience *1*, 451-453.

ACTA BIOL. SZEGED. 33. PP. 105 — 114 (1987)

BIOGEOGRAPHIC, ECOFAUNISTIC AND FLIGHT-DYNAMIC COMPOSITION OF THE APOIDEA FAUNA AT THE TISZA-VALLEY

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(Received: February 15, 1987)

Abstract

During the ten-years studies almost half of the *Apoidea* fauna of the Carpathian basin, a total of 293 species were registered at the Hungarian section of the river Tisza. In scientific respect the *Melitta moczari* TANÁCS, 1985 is a new species. The *Halictus aegyptiacus* FRIESE, 1916; *Lasioglossum opacum* PEREZ, 1895; *Sphecodes ruficornis* SICHEL, 1836; *Spechodes rufiventris* (PANZER, 1798) are new species in the Hungarian fauna. According to zoogeographical estimation the Palaearctic as well as the European species were the most significant. The ratio of the Northern Mediterranean, Holomediterranean, Ponto-Mediterranean and Central European species is considerable.

As to the distribution of the species according to climatic tolerance, the euryoecic eremophilous species have the highest ratio. Based on flight-dynamic estimation the most significant communities were formed by the species with medium flying period.

Introduction

The agricultural development, the concomitant agrotechnique, the use of weedicides were inevitably accompanied by decreasing the food sources, the decrease in the density and species number of the wildbee populations (BENEDEK, 1968b; BANASZAK, 1978). In the course of the past three decades there has been an increase in the significance of studies at areas approaching the natural conditions as well as in the importance of their preservation from chemicals (BANASZAK, 1980). The areas suitable for studies were the dam-system and the flood-plain of the river Tisza, as well as the back area in the vicinity of the safe side, exempt from agroculture. These areas are not treated with pesticides, except for the periodical protection against mosquitos at the holiday resorts along the river Tisza. The majority of the areas are free from continuous human interventions, thus their primary production is almost undisturbed aside from the periodical reaping at the dam sides and flood-plain meadows.

The apidological studies published so far do not comprise an overall, objective analysing method for the judgement of the spread of wildbees. In many cases divergent interpretations exist in respect to the circumscription of the different zoogeographical units. Regarding the diurnal butterflies VARGA (1977) specified an

objective area analytical method. Unfortunately, in case of wildbees, the application of the method is made difficult by the varying degrees of investigation at the different areas, as well as by the contradictions found in the literature. Therefore, the collected wildbees were analysed by the author with the descriptive method, based on the given literary data.

In the Hungarian literature on hymenoptera the zoogeographical spread of the *Hylaeus* species occurring in the Carpathian basin was referred to in detail by M. MÓCZÁR (1960). In case of the species belonging to the *Andrenidae* family, data on such concern are provided by STÖCKERT (1930, 1935, 1942); OSYCHNIUK — PANFILOV — PONOMARJEVA (1978). The latter study also refers to the spread of the *Melittidae* family. As to the *Halictus* and *Lasioglossum* genera, important informative data are given on the zoogeographical spread of the species in the revisory works of EBMER (1969 — 1971).

M. MÓCZÁR (1958) reported on the zoogeographical spread of the species of the *Megachilidae* family. The *Megachilidae* revisory publications from the past two decades — with reference to the zoogeographical spread of the species — are firstly attached to the names of PASTEELS (1965), TKALCU (1967, 1970, 1974a, 1974b, 1974c, 1975), REBMANN (1968) and WARNCKE (1980).

Data on the spread of the *Bombus, Megabombus, Pyrobombus* and *Psithyrus* species have been reported by MÓCZÁR (1953, 1957), REINIG (1976), DELMAS (1976), RASMONT (1983), ŐSBEK (1983). According to the recent taxonomical literary data we are to distinguish the *Anthrophorodae* family. The zoogeographical spread of the species was reported by M. MÓCZÁR (1954, 1957), in case of the species of the *Tetralonia* genus this entry was referred to by TKALCU (1979), and by WARNCKE (1981, 1982) concerning the parasitic wildbees belonging to the *Anthophoridae* family.

The work of PITTIONI and SCHMIDT (1942) deals with the spread and distribution of the *Hymenoptera* with the consideration of environmental, relief relations. In their hymenopterological works, MÓCZÁR (1948) and POSNER (1952) frequently classified the species according to ecological viewpoints. Among the environmental conditions of the wildbee species — apart from the possibilities of feeding and nesting — the climatic factors are of the greatest significance. In case of the wildbees, the author has adapted the conclusions made on several species from the work of PITTIONI and SCHMIDT (1942), but many species were reconsidered, or the unclassified ones arranged into groups. In classifying the species according to climatic tolerance, besides the classic publications, the author made use of his own experiences gained throughout almost one and a half decades (TANÁCS, 1981, 1982; TANÁCS and JÓZAN, 1985), based on his recordings related to the habitat character and climatic tolerance of the species.

In the Hungarian hymenopterological literature the classification of the wildbees according to flight-dynamic principles — concerning the economically appreciable pollinating populations — was published by BENEDEK (1968a, 1968b). In the course of the classification, apart from the author's own observations, other literary data referring to information on phenology and bionomy were also made

use of. According to the reports of KOCOUREK (1966), L. MÓCZÁR and WARNCKE (1972), one part of the *Andrena* species is of medium flying period. In case of the *Halictus* and *Lasioglossum* species the works of M. MÓCZÁR (1967) and EBMER (1969 — 1971) contain important data on flying period. The publication of L. MÓCZÁR and SCHWARZ (1968) comprises data on the flight-dynamics of the species belonging to the *Nomada, Ammobates* and *Paramobatodes* genera of the Carpathian basin. It is apparent from the literature that the different groups also represent a biologically uniform group the flying period of which becomes shorter in general towards Northern direction.

We aimed to determine the composition of the wildbee communities occurring at the studied area, according to zoogeographical spread, climatic tolerance and flight-dynamic distribution.

Material and method

The sampling sites were the grass-communities of the flood-plain and dam-sides between Tiszasziget (165 rkm) and Tarpa (712 rkm) at the Hungarian section of the river Tisza. According to the volumes published under the editorship of J. KAKAS (1960, 1967), author divided the dam-system of the river Tisza into three sections on the basis of the differences in climatic factors. Accordingly, the three reaches were the Lower-Tisza (between Tiszasziget — Tiszaszőlős), the Middle-Tisza (between Kisköre — Tiszafüred) and the Upper-Tisza (between Tokaj — Tarpa).

The plant communities of the studied areas were defined by BODROGKÖZY (1965, 1966). During the sampling wildbees were collected from the following plant-associations:

a) *Agrostio-Alopecuretum pratensis* SOÓ (1933) 1947 *poetosum angustifoliae* BODRG. 1962.
b) *Cynodonto-Poetum-angustifoliae* (RAPAICS, 1926) SOÓ 1957 *alopecuretosum* BODRG. 1966
c) *Cynodonto-Poetum-angustifoliae* (RAPAICS, 1926) SOÓ 1957 *lepidietosum drabae* BODRG. 1966.
d) *Cynodonto-Poetum-angustifoliae* (RAPAICS, 1926) SOÓ 1957 *medicaginetosum rigidulae* BODRG. 1966.
e) *Sclerochloo-Poligonetum avicularis* (GAMS, 1927) SOÓ 1940.
f) *Glycerietum maximae* HUECK, 1931.
g) *Poligono-Bolboschoenetum oeanthetosum* BODRG. 1965.
h) *Salicetum albae-fragilis* ISSLER, 1926. (flower level and grass level)
i) *Alopecuretum pratensis* (NOWINSKI, 1918)
j) *Caricetum gracilis* (GRABNER et HUECK, 1931) TX. 1937.

The wildbees were collected in single from the sampling sites, using butterfly net. The collections lasted for one hour per study site.

The number of collecting days at the three dam-sections was 203 during a period of 10 years, the number of collected and appreciated wildbees was 10016 individuals.

Results and discussion

The data of the species regarding zoogeographical spread, climatic tolerance, flight-dynamics are given in the author's Dissertation for Candidate Degree (TANÁCS, 1986).

Table 1. Distribution of the species at the dam-system and flood-plains of the river Tisza, according to zoogeographical spread

Types according to zoogeographical spread	reaches							
	Along the Lower-Tisza		Along the Middle-Tisza		Along the Upper-Tisza		Along the Tisza river	
	species	%	species	%	species	%	species	%
Holarctic	3	1.31	2	0.94	3	2.34	3	1.02
Palaearctic	48	20.96	47	22.17	37	28.92	57	19.45
Euro-Asian	1	0.44	—	—	1	0.78	2	0.68
Euro-Siberian	9	3.93	6	2.83	3	2.34	12	4.10
Euro-Turanian	1	0.44	1	0.47	2	1.56	2	0.68
Western Palaeartical	9	3.93	10	4.72	6	4.69	13	4.44
European	28	12.23	24	11.32	17	13.29	33	11.26
Holomediterranean	29	12.66	25	11.79	13	10.16	39	13.36
Northern Mediterranean	37	16.16	35	16.51	16	12.50	49	16.72
Ponto-Mediterranean	15	6.55	16	7.55	10	7.81	21	7.17
Ponto-Caspian-mediterranean	5	2.18	6	2.83	2	1.56	6	2.05
Ponto-Caspian	2	0.87	1	0.47	1	0.78	2	0.68
Pontic	6	2.62	6	2.83	3	2.34	8	2.73
Sarmatian	—	—	1	0.47	—	—	1	0.34
Atlantic	5	2.18	1	0.47	1	0.78	5	1.71
Central European	13	5.68	12	5.66	6	4.69	14	4.78
Northern and Central European	6	2.62	6	2.83	3	2.34	7	2.39
Endemic	3	1.31	2	0.94	1	0.78	3	1.02
Unappreciated	9	3.93	11	5.19	3	2.34	16	5.46
Total	229	100.00	212	100.00	128	100.00	293	100.00

Table 2. Distribution of the species at the dam-system and flood-plains of the river Tisza, according to climatic tolerance

Types according to climatic tolerance	reaches							
	Along the Lower-Tisza		Along the Middle-Tisza		Along the Upper-Tisza		Along the Tisza river	
	species	%	species	%	species	%	species	%
stenoecic eremophilous	32	13.97	30	14.15	13	10.16	39	13.31
euryoecic eremophilous	94	41.05	86	40.57	52	40.63	122	41.64
hypereuryoecic intermediary	55	24.02	50	23.58	42	32.81	69	23.55
euryoecic hylophilous	37	16.16	35	16.51	19	14.84	46	15.70
stenoecic hylophilous	9n 2	0.87	1	0.47	—	—	3	1.02
unappreciated	9	3.93	10	4.72	2	1.56	14	4.78
Total	229	100.00	212	100.00	128	100.00	293	100.00

1. APPRECIATION OF THE WILDBEE COMMUNITY ACCORDING TO ZOOGEOGRAPHICAL DISTRIBUTION

A total of 293 bee species were found at the dams and flood-plains of the river Tisza. The Palaeartical and the European species were the most significant fauna elements. Among the Mediterranean distribution types the Northern Mediterranean, Ponto-Mediterranean and Holomediterranean; from the European types the central European species had significant ratios (Table 1).

2. APPRECIATION OF THE WILDBEE COMMUNITY ACCORDING TO CLIMATIC TOLERANCE

At the sampling sites the majority of the community was formed by the euryoecic eremophilous species being fond of heat and showing wider ecological valency. The ratio of the hypereuryoecic intermediary species was considerable (Table 2). Owing to the character of the biotopes, the data unambiguously evidenced the eremophilous species that are fond of heat form the majority of the community at all three reaches. The distribution of the euryoecic hylophilous species within the wildbee community was found to be relatively negligible according to reaches.

Table 3. Distribution of the species at the dam-system and flood-plains of the river Tisza, according to flightdynamics

Types according to flight dynamics	reaches							
	Along the Lower-Tisza		Along the Middle-Tisza		Along the Upper-Tisza		Along the Tisza river	
	species	%	species	%	species	%	species	%
species with short flying period	48	20.96	41	19.34	23	17.97	56	19.11
species with medium flying period	81	35.37	72	33.96	42	32.81	109	37.20
species with continuous and long flying period	24	10.48	24	11.32	20	15.63	28	9.56
bivoltine species with long flying period	64	27.95	63	29.72	39	30.46	84	28.67
unappricated	12	5.24	12	5.66	4	3.13	16	5.46
Total	229	100.00	212	100.00	128	100.00	128	100.00

3. FLIGHT-DYNAMIC APPRECIATION OF THE WILDBEE COMMUNITY

The majority of the community at the sampling sites along the river Tisza was formed by the species with medium flying period. The ratio of the bivoltine species with long flying period was also considerable. The species with short flying period formed about only 1/5 of the community. The wildbee species with continuous and long flying period were found to be the fewest in number within the community (Table 3).

At the studied area, in North-Eastern direction, the number of Mediterranean species showed a decrease, while an increase was detectable in the ratio of the Palaearctic and Ponto-Mediterranean species (Table 1). Summarizing the results, a phenomenon similar to that of experienced by BÁBA (1982) sor snails is observable. The continentality of the species is greater towards North-eastern direction. Upon comparing the results with those obtained from studies performed at the Kiskunság National Park (KNP) (TANÁCS and JÓZAN, 1985), one finds the geographical distribution of the species according to types to be of similar ratios.

There are no fossile material available to be able to prove the distribution of the species (GRÜNWALDT, 1977; EBMER, 1969). The processing of the *Andrena* recens material, forming a significant group of the *Apoideas*, refers to the fact that a large part of the species occurs at the moderately dry, warm, wooded, steppe areas, under optimal living conditions, mainly at the Carpathian basin, in Southern-, South-eastern Europe (GRÜNWALDT, 1977; OSYCHNIUK, 1977). The majority of the species migrated to the area after the glacials, partly from western, but mostly from

the eastern neighbouring refuge areas. Besides the *Andrena* species, those of other genera — like the Eucerae and Tetraloniae — are also the fauna elements originating from the near South-east (M. MÓCZÁR, 1957). Based on the afore-mentioned references it can be assumed that the species of the Carpathian basin of Continental and Southern origin firstly migrated to the area through the East- and South-Carpathian river valleys during the course of the historical times. It seems the primary condition at the time of the Apoidea migration was the favourable climate as well as the continuity of the foster plants.

In respect to the distribution according to climatic tolerance, the different types show a ratio similar to that of observed by the author at the KNP (TANÁCS and JÓZAN, 1985). An essential difference between the types is that the ratio of the stenoecic eremophilous species is 4,75% lower at the Tisza-valley than at the KNP, which can be explained by the lower ratio of mainly the Mediterranean, Ponto-Caspian-Mediterranean and Pontic fauna elements.

The euryoecic eremophilous species that are fond of heat and with wider ecological valency have a ratio similar to that of determined by the author at the area of the KNP.

Appreciating the value within community of the species that are fond of heat at the KNP at the Great Hungarian Plain, at the Tisza-valley and the Hortobágy National Park (HNP), it could be concluded that the highest (60,32%) was found at the KNP (TANÁCS and JÓZAN, 1985) and the lowest (39,2%) at the area of the HNP (TANÁCS, 1981).

Regarding the type of casual spread, this value is 45,6% at the area of the HNP, 23,55% at the Tisza-valley, and only 21,61% at the KNP. The results of the systematic studies carried out at the Lowlands prove that even besides the heterogeneous nature of the sampling sites, the most important community-forming elements are the Southern, South-eastern ones being fond of heat, and mainly the Continental ones having wider ecological valency.

During the flying period the monthly average temperature is 2 — 3 °C lower at the environs of the Upper-Tisza than along the Lower-Tisza reach. At the Tisza-valley, the ratio within community of the species with short as well as medium flying periods shows a continuous decrease towards North-east, according to sampling sections, while an increase is manifest in regard to the bivoltine species and those with continuous and long flying period. This can be explained by the fact that the species with short as well as those with medium flying period are mostly fond of heat, while the bivoltine species and those with continuous and long flying period firstly belong to the type of casual spread.

As to the appreciation of the flight-dynamic types, similar ratios were gained for the Tisza-valley and the KNP (TANÁCS and JÓZAN, 1985). At the HNP the species with short and medium flying periods manifest a lower ratio within community (TANÁCS, 1981). This can partly be explained by the smaller number of species originating from the South. Such are mainly the species with short as well as medium flying periods.

Upon the appreciation of the fauna, summarizing the results it could be concluded that tight relationship may exist between the zoogeographical spread, climatic tolerance as well as flying period; the mathematic estimation of which is an important task of the future.

References

BÁBA, K. (1982): Eine neue zoogeographische Gruppierung der ungarischen Landmollusken und die Wertung des Faunenbildes. — Malacologia *22*, (1—2), 441-454.

BANASZAK, J. (1978): Znaczenie pszczół *(Apoidea)* jako zapylaczy roslin uprawnych. — The importance of bees *(Apoidea)* as pollinators of crop plants. — Wiadomosci ekologiczne *24*, (3), 225-248.

BANASZAK, J. (1980): 22 studies on methods of confusing the numbers of bees *(Hymenoptera, Apoidea)* — Polish Ecological Studies *6* (2), 355-366.

BENEDEK, P. (1986a): A lucernát megporzó vadméhek elszaporitásáról (The breed of wildbees pollinating the alfalfa) — II. Magy. Mezőgazd. *23*(3), 15-16.

BENEDEK, P. (1986b): The flight period of wild bee *(Hymenoptera, Apoidea)* pollinating lucerne, and its plant protection aspects. — Acta phytopath. Hung. *3*, 59-71.

BODROGKÖZY, GY. (1965): Die Vegetation des Theiss-Wellenraumes II. Vegetations und Standart-ökologie der Wasser — und Sumpepflanzenzönosen im Raum von Tiszafüred. — Tiscia *1*, 5-31.

BOGROGKÖZY, GY. (1966): Die Vegetation des Theiss-Wellenraumes III. auf der Schutzdammstrecke zu Szeged durchgeführten Analysen und ihre praktische Bewertung. — Tiscia *2*, 47-67.

DELMAS, R. (1976): Contribution á l'étude de la faune Francoise des *Bombinae (Hymenoptera, Apoidea, Bombidae)*. — Annals. Soc. ent. Fr. (N.S.) *12* (2), 247-290.

EBMER, A.B. (1969—1971): Die Bienen des Genus *Halictus* LATR., s. l. im Grossraum von Linz *(Hymenoptera, Apidae)*. Teil I—III. — Naturkundl. Jb. Stadt Linz. 1969: 133-189., 1970: 19-82., 1971: 63-156.

GRÜNWALDT, W. (1977): Die postglaziale Besiedllung Mitteleuropas durch die Bienengattung *Andrena* F. *(Hymenoptera, Apoidea)*. — VII. Internationales Symposium über Entomofaunistik in Mitteleuropa, Verhandlungen, Leningrad, 206-208.

KAKAS, J. (ed.) (1960): Magyarország éghajlati atlasza (Atlas of climate in Hungary) I. — Akad. Kiadó, Budapest. pp. 78.

KAKAS J. (ed.) (1967): Magyarország éghajlati atlasza (Atlas of climate in Hungary) II. — Akad. Kiadó, Budapest. pp. 263.

KOCOUREK, M. (1966): Prodromus der Hymenopteren der Tschechoslowakei. — Acta faun. ent. natn. Prage *12*, Suppl. *2*, 122.

MÓCZÁR, L. (1948): Die Seehöhe und ökologischen Gesichtspunkte in der Beziehung zoogeographischer Gebietseinheiten. — Fragm. faun. hung. 85-89.

MÓCZÁR, L. and SCHWARZ, M. (1968): A *Nomada-*, *Ammobates-*, *Pasites* és *Parammobatodes* nemek faunakatalógusa (Faunacatalog of the genus *Nomada*, *Ammobates*, *Pasites* and *Parammobatodes)* (Cat. Hym., XXIII). — Folia ent. hung. *21*, 339-360.

MÓCZÁR, L. and WARNCKE, K. (1972): Faunenkatalog der Gattung *Andrena* FABRICIUS (Cat. Hym., XXVI.). — Acta Biol. Szeged. *18*, 185-221.

MÓCZÁR, M. (1953): Magyarország és a környező területek dongóméheinek *(Bombus* LATR.) rendszeres ökológiája. (Ecology of *Bombus* LATR. species living in Hungary and surrounding areas. — Annals. hist.-nat. Mus. natn. hung. *4*, 131-159.

MÓCZÁR, M. (1954): Systematik, Verbreitung und Ökologie der Gattungen *Eucera* LATR. und *Tetralonia* SPIN. *(Hymenoptera)*. — Annals. hist.-natn. Mus. nat. hung. *5*, 368-386.

MÓCZÁR, M. (1957): Méhfélék — Apidae. — In: Magyarország Állatvilága (Fauna Hungariae), XIII. 13. — Budapest, Akad. Kiadó. pp. 76.

MÓCZÁR, M. (1958): Művészméhek — Megachilidae. — In: Magyarország Állatvilága (Fauna Hungariae), XIII. 9. Budapest, Akad. Kiadó. pp. 78.

MÓCZÁR, M. (1960): Ősméhek, földiméhek — *Colletidae, Melittidae* — Magyarország Állatvilága (Fauna Hungariae) XIII. 9. Budapest, Akad. Kiadó. pp. 64.

MÓCZÁR, M. (1967): Karcsúméhek — *Halictidae*. — Magyarország Állatvilága (Fauna Haungariae) XIII. 9. Budapest, Akad. Kiadó, pp. 116.

OSYCHNIUK, A.Z. (1977): Fauna Ukrajna. — Kiev, Naukova Dumka, pp 326.

OSYCHNIUK, A.Z., PANFILOV, D.V. and PONOMARJEVA, A.A. (1978): *Apoidea*. In: Opredelitel naszekomüh evropeijszkij csaszti SzSzSzR. III. Perepancstokrülje. — Red. Medvedeva, Sz. — Leningrad, Nauka. 279-519.

ÖSBEK, H. (1983): Dogu anadolu nun bazi yärelerindeki bombinae *(Hymenoptera, Apoidea, Bombidae)* türleri üzerinde taksonomik ve bazi bioyolojik calismatar — Atatürk Üniverstitesi Yayinlari No.: 621. Ziraat Fakültesi Yayinlari No.: 287. Arastinualar serisi. No.: *188*, 1-70.

PASTEELS, J.J. (1965): Revision des *Megachilidae (Hymenoptera, Apoidea)* de I Afrique noire I les *Geightomella, Chalicodoma* et *Megachili* (s. str.) Mus. Rou. Afr. Cent. Tenuren. — Belg. Ann. (In-80) *137* (I—IX.), 579.

PITTIONI, BR. and SCHMIDT, H. (1942): Die Bienen der südöstlicher Niederdonau. — Niederdonau Natur und Kultur H. *19*, 69. and *24*, 89.

POSNER, M. (1952): Biologisch-ökologische Untersuchungen an Hummeln und ihren Nestern (Veröffentl. Mus. Natur.-, Völker Handelskunde, Bremen *2*, 45-86.

RASMONT, P. (1983): Cataloque commenté des Bourdons de la région Quest-Paléartique *(Hymenoptera, Apoidea)*. — Notes Fauniques de Gemblaux *7*, 3-71.

REBMANN, O. (1968): Beitrag zur Kenntnis der Gattung *Megachile* LATR. *(Hym., Apidae)*: Subgenus *Eutricharaea* und seine bisher bekanntgewordenen Arten. — Deutsche ent. Zeitsch. *15*, 21-48.

REINIG, W.F. (1976): Über die Hummeln und Schmarotzer hummeln von Nordheim — Westfalen *(Hymenoptera, Bombidae)* — Bonn zool. Beitrag. *27*, 267-299.

STÖCKHERT, E. (1930): *Andrena*. In: Schmiedenknecht, Die Hymenopteren Nord- und Mitteleuropas. — Jena, Gustav Fischer Verl. 897-896.

STÖCKHERT, E. (1935): Über einige neue deutsche Arten der *Andrena minutula* — Gruppe *(Hym., Apoid)*. — Deutsche ent. Zeitsch. N. *(1-2)*, 65-85.

STÖCKHERT, E. (1942): Über das Mädchen von *Andrena enslinella* STÖCKH. *(Hym. Apid.)* — Mitteil. Münch. ent. Ger. *32*.N. (2), 572-576.

TANÁCS, L. (1981): The Apoid fauna of the Hortobágy National Park. — In: MAHUNKA, S. (ed.): The fauna of the Hortobágy National Park, Budapest, Akad. Kiadó, 313-320.

TANÁCS, L. (1982): Untersuchung der blumenbesuchenden bienenförmigen Insektenpopulationen *(Hymenoptera: Apoidea)* auf dem Rasen — Ökosystem der Bugacer Sandheide. — Folia ent. hung. *37*, 179-190.

TANÁCS, L. (1986): A Tisza védőtöltés és hullámtér vadméh népességeinek ökológiai viszonyai. (Ecological relations of wildbee communities of the Tisza-dam and flood-plain) — Dissertation for candidate degree, Szeged. pp. 145.

TANÁCS, L. and JÓZAN, ZS. (1985): The Apoid fauna of the Kiskunság National Park. — In: MAHUNKA, S. (ed.): The fauna of the Kiskunság National Park, Budapest, Akad. Kiadó. 401-425.

TKALCU, B. (1967): Bemerkungen zur Taxonomie einiger paläarktischen Arten Familie *Megachilidae (Hymenoptera, Apoidea)*. — Acta ent. bohemos. *64*, 91-104.

TKALCU, B. (1970): Typenrevision der von. J.C. FABRICIUS beschreibenen paläarktischen Arten der Tribus *Osminii (Hymenoptera, Apoidea, Megachilidae)*. — Annotationes Zoologicae et Botanicae *62*, 1-15.

TKALCU, B. (1974a): Bemerkenwerte Bienenfunde in der Tschechoslowakei *(Hymenoptera, Apoidea)*. — Acta ent. bohémoslov. *71*, 205-208.

TKALCU, B. (1974b): Ergebnisse der Albanien — Expeditionen 1961 des Deutschen Entomologischen Institutes. — Beitr. ent. Berlin *24*, (5-8), 323-348.

TKALCU, B. (1974c): Revision und Klassifikation der bisher zur Untergattung, *Hoplosmia* THOMSON gestellten *Anthocopa*- Arten *(Hymenoptera, Apoidea, Megachilidae)*. — Acta ent. bohémoslov. *71*, 114-135.

TKALCU, B. (1975): Revision der europäischen *Osmia (Chalcosmia)*-Arten der *Fulviventris* Gruppe *(Hymenoptera: Apoidea: Megachilidae)*. — Vestnik Ceskoslovenské Spolec. Zool. *39* (4), 297-317.

TKALCU, B. (1979): Revision der europäischen Vertreter der Arten — Gruppe von *Tetralonia ruficornis* (FABRICIUS *(Hymenoptera, Apoidea)*. — Acta Mus. Morav. *64*, 127-152.

VARGA, Z. (1977): Das Prinzip der areal-analitischen Methode in der Zoogeographie und fauna-elemente — Einteilung der europäischen Tagschmetterlinge *(Lepidoptera: Diurna)*. — Acta Biol. Debrecen, *14*, 223-285.

WARNCKE, K. (1980): Die Bienengattung, *Anthidium* FABRICIUS, 1804 in der Westpaläarktis und im turkestanischen Becken. — Entomofauna. *10*, 119-210.

WARNCKE, K. (1981): Die Bienen des Klagenfurter Beckens *(Hymenoptera, Apidae)* — Carinthia, Klagenfurt, *91*, 275-348.

WARNCKE, K. (1982): Zur Systematik der Bienen — Die Unterfamilie *Nomadinae (Hymenoptera, Apoidea)* - Entomofauna. - Z. Entomologie, Linz *3* (8), 97-127.

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DENSITY OF ANT-LION LARVAE *(NEUROPTERA: MYRMELEONTIDAE)* IN SANDY GRASSLANDS

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Abstract

The heteromorph character of Bugacpuszta (Kiskunság National Park, Hungary) is well reflected by the dispersion of the *Euroleon nostras* and the *Myrmeleon inconspicuus* larvae from the 6 species of ant-lion populations demonstrated at the area within 5 years.

The greatest density is found in the *Festucetum vaginatae* typicum association, where the dispersion does not show significant aggregation. In the more closed *Potentillo-Festucetum pseudovinae* association the majority of the *Myrmeleontidae* larvae are localized on mole-hill sides; here the pattern analysis refers to cluster dispersion. The migration activity peak of the quasi sessile predator larvae is in June. The greatest imago density at the area is measurable in July. At the time of oviposition the imagos keep away from the large free sandy areas, because of the rapid decrease in density of their prey and the catch-funnel destructive effect of the wind.

Key words: ant-lion larva, density, season-dynamism

Introduction

One part of the larvae of the ant-lion species *(Myrmeleontidae)* continues sessile predator strategy, catching the prey with their catch-funnel. The space pattern of the larvae living in loose soils greatly depends on the degree of the vegetation coverage, their settlement is connected to smaller-larger free sandy areas.

Their space-pattern has been analysed on several occasions at semi-arid areas (WILSON, 1974; SIMBERLOFF et al. 1978; BOAKE et al. 1983), mostly in respect to the determination of the dispersion type, the probable changes in the prey strategies, as well as regarding studies on group-selective advantage. The majority of these analyses have been performed at places where aggregates are formed by the ant-lion larvae. The season-dynamism and plant-association-dependent density of the *Myrmeleontidae* living at sandy grasslands have not been studied up to this time in Central European relations.

The aims of the present studies were as follows:

1. Determination of larval density depending on vegetation coverage
2. Studies on the space structure of the populations by means of pattern analysis
3. Effect of the number of potential preys on the density of the larvae.

Material and method

The density of the catch-funnel building ant-lion larvae was estimated in 1x1 m squares at the study area at Bugacpuszta of the Department of Zoology of the Attila József University (Szeged, Hungary). With the help of 4x4 sized square net, the pattern analysis of the data can also be performed (following the method of PRÉCSÉNYI, 1981) in case of appropriate funnel number. Two plant-associations were used for the pattern analysis (*Festucetum vaginatae* typicum and *Potentillo-Festucetum pseudovinae*), where the density of the ant-lion larvae is the greatest. During the course of the analysis, the dispersion index ($I = s^2(\bar{x})$ was studied in the function of block size increasing from 625 cm^2 to 1 m^2. The significance was studied at 5% and 10% levels.

The complex synbotanical analysis of the study area was carried out by KÖRMÖCZI et al. (1981; 1982; 1983).

Studies on the season-dynamism of the ant-lion were performed by means of determining the material collected in the years 1983 and 1984 with various trapping methods (Barber-traps, platter- and window-traps, resp.) (GALLÉ et al. 1985). In certain cases the larvae change their catch-funnels, when — though in small number — they can be collected by soil traps.

The density of the potential preys of the *Myrmeleontidae* was studied at the continuous free sandy areas with cylindrical (diameters of 23 and 32 mm) ethylene-glycol traps digged into the ground. 50 — 50 traps were placed at two 1,5x20 m sized sand-zones, in parallel rows (distances of 25 cm) to their edges covered with vegetation. The trapped animals were defined after 3 days. The groups which cannot be caught by the *Myrmeleontidae* larvae (*Acrididae*, large *Carabidae*, *Cicindelidae*) were left unconsidered.

Results

DENSITY OF VEGETATION COVERAGE-DEPENDENT ANT-LION LARVAE

During the course of 5 years' collection 6 ant-lion species were found at the study area: *Acanthaclisis occitanica* VILL., *Distoleon tetragrammicus* FABR. *Creoleon plumbeus* OLIV., *Myrmecaelurus trigrammus* PALL., *Myrmeleon inconspicuus* RAMB., *Euroleon nostras* FOURC. The first two species are rather rare at the area.

The density studies were performed on the populations of the two most frequent catch-funnel-building species, the *Euroleon nostras* and the *Myrmeleon inconspicuus*. The average funnel-density related to the whole study area was 0,52/m^2. The largeness of the plant-associations at the enclosed 2,3 ha sized sandy grassland (on the basis of a botanical record from 1983), as well as the density values per association of the catch-funnel-building ant-lions are demonstrated on Table 1.

The sample area withdrawn from grazing 10 years ago does not completely represent the vegetation coverage relations of the several thousand ha sized Bugacpuszta. On the effect of intensive grazing — as considerable degradation — the expansion of the open grasses of importance from the viewpoint of the ant-lion larvae (*Festucetum vaginatae* typicum) greatly decreased at the largest part of the puszta; shrinking to 1 — 200 m^2 sized continuous stands according to KÖRMÖCZI (1982). Their places are generally occupied by the closed *Cynodon dactylon* facies, where no funnel-building *Myrmeleontidae* larvae live.

Table 1. Density-dependence of funnel-building ant-lions on plant-associations (combined data of the surveys of 29—30 August, 1984 and 15 September, 1984)

Plant-association	Plant coverage		Larval density ind/m²	Total number of ant-lions
	%	total m²		
Festucetum vag. typicum	12.56	2888.8	2.1	6066
Molinio-Salicetum rosmarinifoliae	22.47	5168.1	0	0
Potentillo-Fest. pseudovinae	56.53	13001.9	0.44	5721
its facies:				
Cynodon dactylon	1.67	384.1	0	0
Euphorbia seguieriana	0.13	29.9	0.35	10
Stipa capillata	2.39	549.7	0	0
Bromus squarrosus	0.23	52.9	0.25	13
Carex liparicarpos	3.57	821.1	0.1	82
Holoschoenus vulgaris	0.2	46.0	0	0
Salix rosmarinifolia	0.28	64.4	0	0
				Σ: 11892

Table 2. Season-dynamism of the ant-lions collected by various trapping methods (l = larva, i = imago individual number)

1983	individual number in the different months													
	4.		5.		6.		7.		8.		9.		10.	
	l.	i.	l.	i.	l.	i.	l.	i.	l.	i.	l.	i.	l.	i.
Barber-trap	5		7	—	32	—	7	—	1	—	5	—	—	—
Platter-trap	1	—	10	—	27	—	5	2	—	1	—	3	—	—
Window-trap	—	—	—	—	1	1	—	13	—	6	—	—	—	—
1984														
Barber-trap	1		1		4	—	1	—	—	—	—	—	1	—

Sampling was performed following oviposition (29—30 August and 15 September, 1983, resp.), at the time when the number of hatched juveniles is high.

As the consequence of considerable juvenile mortality, there is a striking decrease in incidence with the growth of larval size (SIMBERLOFF et al. 1978). Therefore, the estimation of density at the beginning of Summer would give a much lower value.

Owing to their occasional change of place, the larvae are collectable — though in small number — with soil traps. It is apparent from the analysis of the distribution of the ant-lions caught with various trap types in the years 1983 and 1984 (Table 2, Figs. 1, 2) that the "locomotive peak" of the larvae is in June, the period prior to pupation. This is caused by the acceleration of the metabolism due to the heat and the enchanced need of food owing to the increase of body size, which is manifested in the longer migration distances as well. In July, because of maturation, there is a strong decrease in larval number. In case of fine weather, another larval locomotion (but of slighter degree) is observable around September—October, however, the participants of this are mostly the hatched juveniles. The first imagos appear in June, the peak is in July, then there is a gradual decrease in their number till September.

PATTERN ANALYSIS

According to the results of the recording methods used for the estimation of the pattern analysis (based on 122 catch-funnels) significant clustering was not at all observable in the *Festucetum vaginatae* typicum association (Fig. 3). This may be due to the more or less even dispersion of the *Festuca vaginata* roots; in their typical association the bare sandy areas betwixt — which are the potential habitats for the ant-lion larvae — can quasi be regarded as uniform. This result would presumedly not be considerably modified neither by higher sampling quadrate number, nor by the even more dense quadratic division.

In the *Potentillo-Festucetum pseudovinae* association, the density of the *Myrmeleontidae* larvae is much lower — almost the fifth — as compared to the density values measured for the *Festucetum vaginatae* typicum. Therefore, not every recording by 1x1 m sized quadrates divided into 16 equal parts, used for the density-estimation sampling, was suitable for estimation from the viewpoint of pattern analysis. According to the calculations (Fig. 4), significant aggregation of the ant-lion larvae could be experienced in the *Potentillo-Festucetum pseudovinae* association in case of block size 4 (50 x 50 cm). This is a good verification of the observation performed in nature that at the study area of Bugacpuszta, at several places the mole living there (*Talpa europea* L.) breaks up with its digging the coverage of mainly the *Potentillo-Festucetum* association, growing on sandy-hill ridges and on the whole regarded as closed. At the cc. quarter m^2 sized free sandy areas developing in such manner, 20—30 small ant-lion funnels are often detectable at the end of Summer.

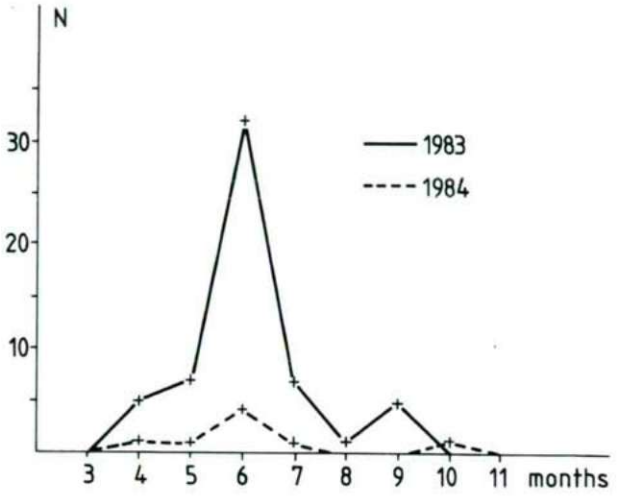

Fig. 1. Season-dynamism of ant-lion larvae collected with Barber-traps.

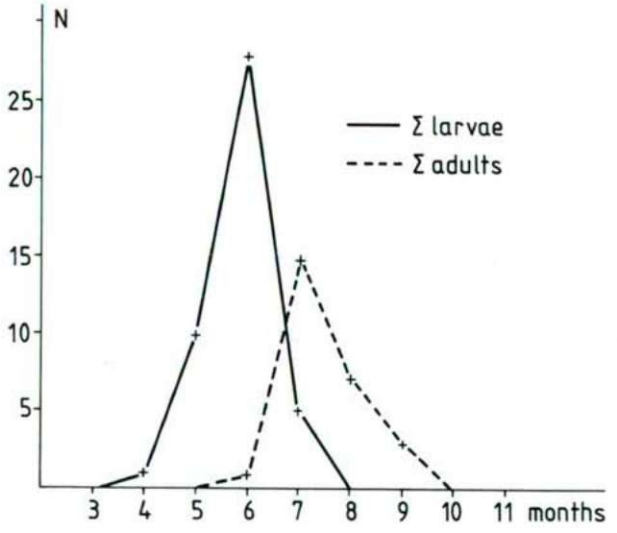

Fig. 2. Season-dynamism of ant-lions based on the global material of the platter- and window-traps.

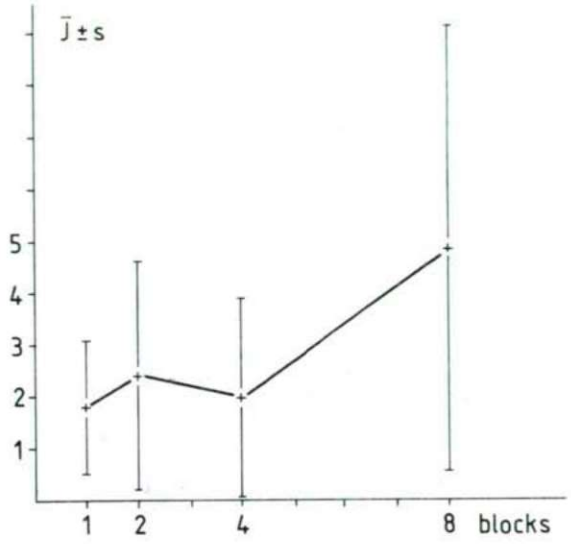

Fig. 3. Results of the pattern-analysis regarding the *Festucetum vaginatae* typicum association.

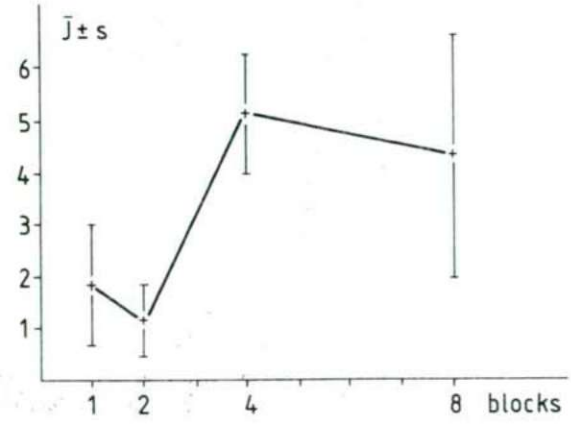

Fig. 4. Results of the pattern-analysis regarding the *Potentillo-Festucetum pseudovinae* association.

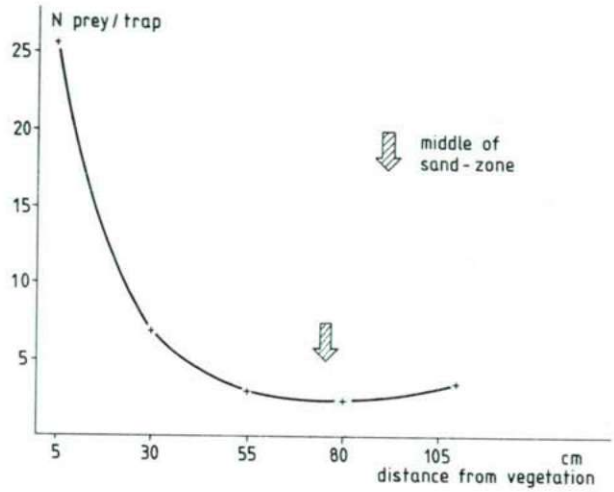

Fig. 5. Potential prey relations of the large free sandy areas in the function of distance from vegetation, based on Tables 1. and 3.

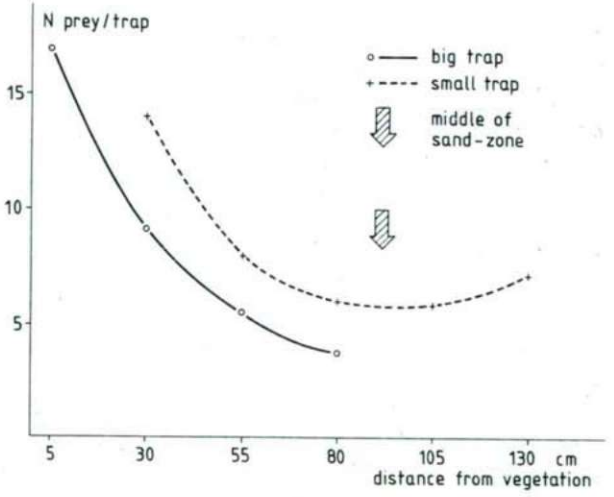

Fig. 6. Potential prey relations of the large free sandy areas in the function of distance from vegetation, based on Tables 2. and 4.

Table 3. Relationship between the incidence of prey experienced at the continuous free sandy areas and the distance from the edge of the sandy surface I.
25—28 June, 1985. forest-zone
(The 1. row is beside the vegetation).

Prey taxon	Dominance % in the different rows				
	1	2	3	4	5
Formicoidea	82.0	23.9	11.1	9.1	29.0
other *Hymenoptera*	—	—	3.7	4.5	—
Collembola	9.8	44.7	22.2	27.2	19.4
Coleoptera	1.0	10.4	29.6	22.7	19.4
Diptera	2.4	4.5	11.1	13.6	9.7
Homoptera	2.0	3.0	3.7	—	3.2
Heteroptera	0.4	1.5	—	—	—
Araneidea	0.4	3.0	—	9.1	3.2
Aphidina	—	4.5	3.7	9.1	16.1
Acariformes	—	5.5	3.7	—	—
Staphylinidae	—	—	11.1	4.5	—
Number of preys referring to 1 trap	25.5	6.7	3.0	2.4	3.4

EFFECT OF THE NUMBER OF POTENTIAL PREYS ON THE DENSITY OF THE LARVAE

The qualitative and quantitative estimation of the potential ant-lion larval preys collected from trappings performed at Bugacpuszta and the sand-zone of a clearing (Tables 3, 4., Figs. 5, 6) indicates why there is no increase in the number of *Myrmeleontidae* larvae at large free sandy areas.

At the time of the first study series (25—28 June, 1985) the majority of the traps at the puszta were covered with sand by the wind, thus this recording could not be evalueted. Nevertheless, it also follows from this that the continuous, large free sandy areas are not advantageous for the ant-lion larvae even for energetic point of view either, as to restore the catch-funnels at these sites plenty of time and energy would be required. The repetitions of the study were appreciable. As observable in Fig. 5 and 6, the number of preys is the lowest at the middle of the 1,5 m wide sand-zone.

According to the results, the number of potentially reckoned prey for the ant-lion larvae shows rapid decrease moving away from the vegetation-covered edges of the sand-zone. Therefore it is firstly due to this and the strongly destructive effect of the wind that at the time of oviposition the *Myrmeleontidae* avoid the open free sandy areas.

Table 4. Relationship between the incidence of prey experienced at the continuous free sandy areas and the distance from the edge of the sandy surface II.
23—26 July, 1985. Puszta
(k = small, n = large trap diameter)

Prey taxon	Dominance % in the different rows									
	1		2		3		4		5	6
	n.	k.	n.	k.	n.	k.	n.	k.	k.	k.
Formicoidea	70.5	6.4	40.5	62.5	22.7	22.6	20.0	12.5	12.7	15.8
Mutillidae	—	—	2.7	—	—	—	—	8.3	—	3.5
Sphecoidea	—	—	—	—	—	—	—	—	—	3.5
other *Hymenoptera*	2.9	3.2	2.7	5.7	9.1	12.9	13.3	—	6.4	15.7
Staphylinidae	—	—	—	—	—	—	—	—	2.1	—
other *Coleoptera*	8.8	25.8	5.4	5.4	13.6	12.9	13.3	20.8	27.6	26.3
Diptera	2.9		2.7	5.4	13.6	3.2	26.6	12.5	12.7	19.3
Heteroptera	4.4	6.4	5.4	5.4	27.2	6.4	6.7	29.2	14.9	7.0
Acariformes	7.3	35.5	8.1	14.3	13.6	22.5	13.3	12.5	12.8	8.8
Blattidea	1.5	—	—	—	—	3.2	—	—	—	—
Araneidea	1.5	3.2	2.7	—	—	3.2	—	—	2.1	—
Collembola	—	9.7	30.0	—	—	9.7	—	4.2	—	—
Acrididae juv. larvae	—	6.4	—	—	—	—	6.7	—	2.1	—
Myrmeleontidae larvae	—	3.2	—	—	—	—	—	—	—	—
Homoptera	—	—	—	1.8	—	3.2	—	—	—	—
Isopoda	—	—	—	—	—	—	—	—	2.1	—
Aphidina	—	—	—	—	—	—	—	—	2.1	—
Microlepidoptera	—	—	—	—	—	—	—	—	2.1	—
Average number of preys referring to 1 trap	17	7.75	9.27	14	5.5	7.75	3.75	6.0	5.87	7.1

References

BOAKE, C.R.B., ANDOW, D. and VISSCHER, P.K. (1984): Spacing of Ant-lions and Their Pits. — The American Midland Naturalist, *111*, 192-194.

GALLÉ, L., HORNUNG, E., SZŐNYI, G., GYŐRFFY, GY. and KINCSEK, I. (1985): A JATE Állattani Tanszék komplex ökológiai kutatása, in: TÓTH, K. (col.): Tudományos kutatások a Kiskunsági Nemzeti Parkban 1975—1984 (Complex ecological studies in a sandy grassland in Kiskunság National Park) — Hungexpo, Budapest, 174-197.

KÖRMÖCZI, L., BODROGKÖZY, GY. and HORVÁTH, I. (1981): Investigation of biological production and bioclimate of sandy grasslands in Bugac (Great Hungarian Plain between Danube and Tisza). — Acta Biol. Szeged. *27*, 55-69.

KÖRMÖCZI, L. (1982): A felsőbugacpusztai homoki gyeptársulások synökológiai analízise (Synecologic analysis of the sandy grassland communities in Felső-Bugacpuszta). Szeged (Thesis).

KÖRMÖCZI, L. (1983): Correlations between the zonation of sandy grasslands and the physico-chemical condition of their soil in Bugac. — Acta Biol. Szeged. 29, 117-127.

PRECSÉNYI, I. (1981): Mintázat (pattern) analizis. in: HORTOBÁGYI, T. and SIMON, F. (eds.): Növényföldrajz, társulástan és ökológia. — Tankönyvkiadó, Budapest, 208-210.

SIMBERLOFF, D., KING, L., DILLON, P., LOWRIE, S., LORENCE, D. and SCHILLING, E. (1978): Holes in the doughnut theory: the dispersion of ant-lions. Brenesia, 14-15, 13-46.

WILSON, D.S. (1974): Prey capture and competition in the ant-lion. — Biotropica, 6, 187-193.

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CHECK LIST OF *AUCHENORRHYNCHA* ON A SANDY GRASSLAND IN THE KISKUNSÁG NATIONAL PARK (HUNGARY)

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Abstract

183 species of 10 *Auchenorrhyncha* families were defined on a sandy grassland in the Kiskunság National Park between the years 1976 — 1986. This paper comprises the check list.
Key words: Auchenorrhyncha, check list, grassland

Complex ecological studies are being performed since 1976 at the Bugacpuszta region of the Kiskunság National Park (KNP) (Hungary) (MÓCZÁR et al. 1980). In the course of the past ten years, an almost complete check list could be compiled of the species occurring at the region. Contrary to the 94 species demonstrated hitherto (GYÖRFFY, 1982), a close to twofold increase in species number is manifested (183). Since the recently published first volume of a book dealing with the fauna of the KNP (MAHUNKA, 1986) does not contain the *Cicadinea* group, author finds it useful to make available his data collected up till now.

The collections are from the Bugacpuszta grass-cenoses of the KNP. The detailed description of the area can be found in earlier publications (KÖRMÖCZI et al. 1981; KÖRMÖCZI, 1983). The collections were mainly performed with traps: suction trap, Barber trap, ethylene-glycol platter- and window-traps. The first two have already been reviewed (GYÖRFFY, 1982). The platter-traps are 15 cm in diameter, the window-traps are 50x25 cm in size, placed in 3 heights (ground level, 50 cm and 100 cm). The considerable increase in species number was mainly owing to the window-traps, presumably by means of the species coming from surrounding areas during the course of migration.

A total of 183 species belonging to 10 families could be defined; *Cicadellidae:* 125 species, *Delphacidae:* 37 species, *Tettigometridae:* 8 species, *Cercopidae:* 7 species and 1 — 1 species from 6 further families.

The detailed check list is given in the followings:

Familia:*Cicadellidae*

Aconurella quadrum HERRICH-SCHÄFFER, 1838
Agallia laevis RIBAUT, 1935
Allygus atomarius FABRICIUS, 1794
Allygus commutatus FIEBER, 1872

Allygus mixtus FABRICIUS, 1794
Anaceratagallia ribauti OSSIANNILSON, 1938
Anoscopus albiger GERMAR, 1821
Anoscopus histrionicus FABRICIUS, 1794
Anoscopus serratulae FABRICIUS, 1775
Aphrodes bicinctus SCHRANK, 1776
Arboridia parvula BOHEMAN, 1845
Arocephalus languidus FLOR, 1861
Arthaldeus pascuellus FALLÉN, 1826
Arthaldeus striifrons KIRSCHBAUM, 1868
Artianus interstitialis GERMAR, 1821
Athysanus argentarius METCALF, 1955
Austroagallia sinuata MULSANT-REY, 1855
Balclutha punctata FABRICIUS, 1775
Balclutha rhenana WAGNER, 1939
Batracomorphus allionii TURTON, 1802
Batracomorphus irroratus LEWIS, 1834
Bobacella corvina HORVÁTH, 1903
Chlorita dumosa RIBAUT, 1933
Chlorita hungarica RIBAUT, 1933
Chlorita paolii OSSIANNILSON, 1939
Cicadella viridis LINNÉ, 1758
Cicadula frontalis HERRICH-SCHÄFFER, 1835
Cicadula placida HORVÁTH, 1897
Cicadula quadrinotata FABRICIUS, 1794
Deltocephalus pulicaris FALLÉN, 1806
Dikraneura similis EDWARDS, 1885
Dikraneura variata HARDY, 1850
Doratura exilis HORVÁTH, 1903
Doratura heterophyla HORVÁTH, 1903
Doratura homophyla FLOR, 1861
Doratura impudica HORVÁTH, 1897
Doratura stylata BOHEMAN, 1847
Dryodurgades dlabolai WAGNER,
Edwardsiana candidula KIRSCHBAUM, 1868
Edwardsiana rosae LINNÉ, 1758
Emelyanoviana mollicula BOHEMAN, 1845
Errastunus notatifrons KIRSCHBAUM, 1868
Erythroneura discolor HORVÁTH, 1897
Eupelix cuspidata FABRICIUS, 1775
Eupteryx aurata LINNÉ, 1758
Eupteryx calcarata OSSIANNILSON, 1936
Eupteryx collina FLOR, 1861
Eupteryx notata CURTIS, 1837
Eupteryx ornata LETHIERRY, 1874
Eupteryx stachydearum HARDY, 1850
Eupteryx thoulessi EDWARDS, 1926
Euscelidius schenckii KIRSCHBAUM, 1868
Euscelis incisus KIRSCHBAUM, 1858
Evacanthus acuminatus FABRICIUS, 1794
Goniagnathus brevis HERRICH-SCHÄFFER, 1835
Graphocraerus ventralis FALLÉN, 1806
Handianus ignoscus MELICHAR, 1896

Hardyopsis insularis LINDBERG, 1948
Hecalus glaucescens FIEBER, 1866
Hephathus freyi FIEBER, 1868
Hephathus nanus HERRICH-SCHÄFFER, 1836
Iassus lanio LINNÉ, 1758
Idiocerus albicans KIRSCHBAUM, 1868
Idiocerus decimusquartus SCHRANK, 1776
Idiocerus distinquendus KIRSCHBAUM, 1868
Idiocerus impressifrons KIRSCHBAUM, 1868
Idiocerus nobilis FIEBER,
Idiocerus populi LINNÉ, 1758
Idiocerus ustulatus MULSANT-REY, 1855
Jassargus obtusivalvis KIRSCHBAUM, 1868
Jassargus sursumflexus THEN, 1902
Kybos abstrusa LINNAVUORI, 1949
Limotettix striola FALLÉN, 1806
Limotettix transversus FALLÉN, 1826
Linnavuoriana sexmaculata HARDY, 1850
Macropsis cerea GERMAR, 1837
Macropsis impura BOHEMAN, 1847
Macropsis vestita RIBAUT, 1952
Macrosteles fieberi EDWARDS, 1889
Macrosteles laevis RIBAUT, 1927
Macrosteles quadripunctulatus KIRSCHBAUM, 1868
Macrosteles sexnotatus FALLÉN, 1806
Macrosteles sordidipennis STÅL, 1858
Macustus grisescens ZETTERSTEDT, 1828
Megophtalmus scanicus FALLÉN, 1806
Mendrausus pauxillus FIEBER, 1869
Metalimnus formosus BOHEMAN, 1845
Micantulina stigmatipennis MULSANT-REY, 1855
Mocuellus collinus BOHEMAN, 1850
Mocuellus metrius FLOR, 1861
Mocydia crocea HERRICH-SCHÄFFER, 1837
Mocydiopsis attenuata GERMAR, 1821
Mocydiopsis parvicauda RIBAUT, 1939
Neoaliturus fenestratus HERRICH-SCHÄFFER, 1834
Neoaliturus haematoceps MULSANT-REY, 1855
Paluda preyssleri HERRICH-SCHÄFFER, 1838
Paluda vitripennis FLOR, 1861
Paralimnus phragmitis BOHEMAN, 1847
Paramesus obtusifrons STÅHL, 1853
Penthimia nigra GOEZE, 1778
Pinumius areatus STÅHL, 1858
Planaphrodes elongatus LETHIERRY, 1876
Platymetopius major KIRSCHBAUM, 1868
Platymetopius undatus DE GEER, 1773
Psammotettix alienus DAHLBOM, 1850
Psammotettix cephalotes HERRICH-SCHÄFFER, 1834
Psammotettix confinis DAHLBOM, 1850
Psammotettix hungaricus OROSZ, 1981
Psammotettix pallidinervis DAHLBOM, 1850
Psammotettix provincialis RIBAUT, 1925

Psammotettix slovacus DLABOLA, 1948
Recilia schmidtgeni WAGNER, 1939
Speudotettix subfusculus FALLÉN, 1806
Stictocoris picturatus C. SAHLBERG, 1842
Streptanus aemulans KIRSCHBAUM, 1868
Streptanus sordidus ZETTERSTEDT, 1828
Stroggylocephalus livens ZETTERSTEDT, 1840
Tetartostylus pellucidus WAGNER, 1951
Turrutus socialis FLOR, 1861
Ulopa lugens GERMAR, 1821
Ulopa trivia GERMAR, 1821
Zygina nivea MULSANT-REY, 1855
Zygina tithide FERRARI, 1882
Zyginidia pullula BOHEMAN, 1845

Familia: *Delphacidae*

Acanthodelphax spinosa FIEBER, 1866
Anakelisia perspicillata BOHEMAN, 1845
Criomorphus borealis J. SAHLBERG, 1871
Delphacodes albifrons FIEBER, 1879
Delphacodes venosus GERMAR, 1830
Dicranotropis hamata BOHEMAN, 1847
Ditropsis flavipes SIGNORET, 1865
Euconomelus lepidus BOHEMAN, 1847
Eurybregma nigrolineata SCOTT, 1875
Eurysa clypeata HORVÁTH, 1897
Eurysa lineata PERRIS, 1857
Eurysula lurida FIEBER, 1866
Falcotoya minuscula HORVÁTH, 1897
Gravesteiniella boldi SCOTT, 1870
Hyledelphax elegantulus BOHEMAN, 1847
Jassidaeus lugubris SIGNORET, 1865
Javesella dubia KIRSCHBAUM, 1868
Javesella pellucida FABRICIUS, 1794
Kelisia brucki FIEBER, 1878
Kelisia guttula GERMAR, 1818
Kelisia irregulata HAUPT, 1935
Kelisia monoceros RIBAUT, 1934
Kelisia pallidula BOHEMAN, 1847
Kelisia perrieri RIBAUT, 1934
Kelisia ribauti WAGNER, 1938
Kelisia vittipennis J. SAHLBERG, 1868
Kosswigianella exiqua BOHEMAN, 1847
Megadelphax sordidulus STÅL,1853
Metadelphax propinqua FIEBER, 1866
Muellerianella fairmairei PERRIS, 1857
Muirodelphax aubei PERRIS, 1857
Ribautodelphax albostriata FIEBER, 1866
Ribautodelphax imitans RIBAUT, 1953
Stenocranus minutus FABRICIUS, 1787
Struebingianella palliceps HORVÁTH, 1897
Weidnerianella marginata FALLÉN, 1826
Xanthodelphax straminea STÅL, 1858

Familia: *Tettigometridae*

Tettigometra atra HAGENBACH, 1825
Tettigometra concolor FIEBER, 1865
Tettigometra depressa FIEBER, 1865
Tettigometra impressopunctata DUFOUR, 1846
Tettigometra macrocephala FIEBER, 1865
Tettigometra sordida FIEBER, 1865
Tettigometra sulphurea MULSANT-REY, 1855
Tettigometra virescens PANZER, 1799

Familia: *Cercopidae*

Aphrophora alni FALLÉN, 1805
Aphrophora salicina GOEZE, 1778
Lepyronia coleoptrata LINNÉ, 1758
Neophilaenus campestris FALLÉN, 1805
Neophilaenus lineatus LINNÉ, 1758
Neophilaenus minor KIRSCHBAUM, 1868
Philaenus spumarius LINNÉ, 1758

Familia: *Tropiduchidae*

Trypetimorpha fenestrata COSTA, 1862

Familia: *Issidae*

Ommatidiotus dissimilis FALLÉN, 1806

Familia: *Achilidae*

Cixidia marginicollis SPINOLA, 1839

Familia: *Membracidae*

Stictocephala bisonia KOPP et YONKE, 1977

Familia: *Cixiidae*

Pentastiridius leporinus LINNÉ, 1761

Familia: *Dictyopharidae*

Chanithus pannonicus GERMAR, 1830

References

GYÖRFFY, GY. (1982): *Auchenorrhyncha* of a sandy soil mosaic-grassland: quantitative relations, bionomic and ecological valence data. — Fol. Ent. Hung. *43*, 43-54.

KÖRMÖCZI, L., BODROGKÖZY, GY. and HORVÁTH, I. (1981): Investigation of biological production and bioclimate of sandy grasslands in Bugac (Great Hungarian Plain between Danube and Tisza) — Acta Biol. Szeged. *27*, 55-69.

KÖRMÖCZI, L. (1983): Correlation between the zonation of sandy grasslands and the physico-chemical condition of their soil in Bugac — Acta Biol. Szeged. *29*, 117-127.

MAHUNKA, S. (ed.) (1986): The fauna of the Kiskunság National Park. Vol. 1. — Akadémiai K., Budapest. 1-491.

MÓCZÁR, L., GALLÉ, L., GYÖRFFY, GY. and H. HORNUNG, E. (1980): Complex ecological investigations in a sandy soil grassland: aims and general methodology — Acta Biol. Szeged. *26*, 161-164.

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HUMANBIOLOGICAL COMPARISON OF THE SOMATIC DEVELOPMENT OF SECONDARY SCHOOL STUDENTS AND STUDENTS TRAINED FOR SKILLED WORK

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Abstract

A comparison is given the means of the body height, body weight, normal chest circumference of 12831 secondary school students and 6200 students trained for skilled work. It is determined that the arithmetic mean are lower, as demonstrable statistically, in 53.3 p.c. of the age groups of the boys, and 20 p.c. of the girls receiving vocational training, as compared with those of the secondary school students.

In case of the girls attending both types of school, the frequency of irregular menstruation cycle is equally around 5 — 6 p.c. in the 4 — 5th year following the menarche.

64.2 p.c. of the girls attending secondary school, and only 43.8 p.c. of the girls trained for skilled work discussed sexual problems with their parents; their sexual education is not satisfactory.

Key words: somatic development, students trained for skilled work, secondary school students.

Introduction

In a former publication (FARKAS et al. 1983) a detailed report was given about a survey aiming at studies on the puberty of Hungarian girls. Within the frame of these studies it was possible to measure the body height, body weight and normal chest circumference of the youths.

One part of the studied youths is being trained for skilled work, the other part attends grammar school or specialized secondary school (hereinafter: secondary school). This provided possibility for comparing the somatic development and a few characteristic criteria of puberty of the students attending the two different types of school.

The comparison is also accounted for by the fact that there are only few subsequent data on Hungarian students trained for skilled work.

The studies published in the 60s and 70s mainly aimed at the examination of muscularity (BUGYI, 1965, 1966, 1971 — 72; BUGYI and LŐRINCZ, 1966), or gave analysis of productivity (BUGYI, 1971) and the connections between the circulatory organ system and burden, resp. (BUGYI, 1971a). The study on body composition also belongs to this (BUGYI, 1971b).

In a study published in 1976 involving six body measurements of students trained for skilled work, the somatic development was found to be satisfactory in general (GIRINYI et al. 1977).

Eiben compared the somatic development of 199 Budapest students receiving training for work in chemical industry with the Budapest data of 1968/69 (EIBEN et al. 1971, 1979).

In a recently published report (BAKONYI, 1984) the differences in the somatic characters of 7464 secondary school students and 3088 students trained for skilled work are analysed.

There are deviations in respect to the final conclusions drawn in the cited publications. Certain authors did not find essential differences between the somatic development of the students attending the two types of school (GIRINYI et al. 1977, EIBEN et al. 1979), while observations made on a larger sample led to opposite conclusions (BAKONYI, 1984).

On the basis of them, it does not seem unnecessary to report on our experiences gained in connection with the measurements deriving from the beginning of the 80s from this point of view as well.

Material and method

In the years 1981 — 1984 3677 boys and 2367 girls trained for skilled work, and 3144 boys and 9395 girls attending secondary schools were studied. The dissimilar ratio of the two sexes is partly related to the trend of vocational training, partly to our original objective (studies on menarche). The observations concerning the somatic development of the students attending the two types of school can be comprehended as the "secondary product" of our original objective.

Table 1. The more important parameters of the body weight of boys trained for skilled work (n_1) and those attending grammar school and specialised secondary school (n_2), according to half-year age-groups

Age	Boys trained for skilled work			Boys attending grammar school and specialised secondary school		
	n_1	$\bar{x}_1$	s_1	n_2	$\bar{x}_2$	s_2
14.0	126	51.23	10.15	34	56.73	12.42
14.5	549	54.29	11.20	303	56.28	9.95
15.0	535	56.74	10.90	450	58.42	10.20
15.5	613	59.75	11.28	420	59.94	9.39
16.0	581	60.82	10.28	416	62.82	9.62
16.5	535	63.82	11.40	368	64.46	10.26
17.0	430	64.11	11.00	404	65.79	9.06
17.5	166	65.16	10.39	357	65.89	9.35
18.0	102	64.21	8.99	306	68.37	9.59
18.5	40	64.60	8.13	86	66.12	9.24
Total:	3677			3144		

In the course of the measurements the introductions of Martin were followed (MARTIN and SALLER, 1956). The body height and normal chest circumference were measured with mm, the body weight with 50 g accuracy.

The information on puberty were collected by means of questionnaires.

The evaluations were obtained with R-55 type computer by the co-workers of the László Kalmár Cybernetics Laboratory of the Attila József University, Szeged.

The results are summarized in Tables 1.—6., with the arithmetic means of the three body measurements given according to sex, age groups and types of school. The elemental numbers indicated in the Tables are not always identical in case of the three characters, since not every student could be measured.

In our evaluation the arithmetic means ($\bar{x}$) and standard deviation (s) were also calculated, on the basis of its using the two-sample Student's test it was examined whether the differences in arithmetic means could be verified according to character between the two types of students of the same age and sex. The probability level for our statements was chosen 95 p.c.

Results

1. SOMATIC DEVELOPMENT

Though differences between the arithmetic means are manifested in several age groups regarding both sex and all three characters, these are not significant in every case. It could be determined, however, that from the studied 10 age groups (between age 14—18.5), the body weight was found to be lower in 6, the body height in 9, and the normal chest circumference in 1 age group(s) of the boys trained for skilled work, as compared to the secondary school students. These 16 cases correspond to 53.3 p.c. of all the chances (30 in case of the 3 characters of the 10 age-groups).

Table 2. The more important parameters of the body height of boys trained for skilled work (n_1) and those attending grammar school and specialised secondary school (n_2), according to half-year age-groups

Age	Boys trained for skilled work			Boys attending grammar school and specialised secondary school		
	n_1	$\bar{x}_1$	s_1	n_2	$\bar{x}_2$	s_2
14.0	126	163.32	7.56	34	166.44	8.12
14.5	549	165.07	8.23	303	167.72	8.48
15.0	535	167.18	8.15	450	169.50	7.26
15.5	613	170.10	7.16	420	171.46	7.12
16.0	581	170.91	7.10	416	172.96	6.65
16.5	535	172.54	6.68	368	174.19	6.43
17.0	430	171.89	6.65	404	175.06	6.32
17.5	166	173.05	6.71	357	174.99	6.28
18.0	102	173.89	7.11	306	176.01	6.71
18.5	40	173.48	6.54	86	175.06	6.68
Total:	3677			3144		

In respect to the girls trained for skilled work, lower values were only found for the body height arithmetic means in 6 cases, while their normal chest circumference was found to be higher than the means obtained for the secondary school students in case of two age-groups. Accordingly, the means found for the girls trained for skilled work were lower in only 20 p.c. of the cases compared to the girls attending secondary school.

Table 3. The more important parameters of the normal chest circumference of boys trained for skilled work (n_1) and those attending grammar school and specialised secondary school (n_2), according to half-year age-groups

Age	Boys trained for skilled work			Boys attending grammar school and specialised secondary school		
	n_1	$\bar{x}_1$	s_1	n_2	$\bar{x}_2$	s_2
14.0	126	79.61	7.07	34	81.85	7.68
14.5	548	81.29	7.55	303	82.05	6.94
15.0	534	83.07	7.11	449	83.89	6.96
15.5	613	85.41	7.29	420	85.08	6.35
16.0	581	86.23	6.63	416	86.98	6.37
16.5	535	88.49	7.50	368	87.95	6.80
17.0	430	88.89	7.01	404	89.34	6.10
17.5	166	89.73	7.04	357	89.51	6.03
18.0	102	89.19	5.78	306	90.51	5.85
18.5	40	90.28	6.01	86	90.24	5.81
Total:	3675			3143		

Table 4. The more important parameters of the body weight of girls trained for skilled work (n_1) and those attending grammar school and specialised secondary school (n_2), according to half-year age-groups

Age	Girls trained for skilled work			Girls attending grammar school and specialised secondary school		
	n_1	$\bar{x}_1$	s_1	n_2	$\bar{x}_2$	s_2
14.0	25	159.57	5.51	115	159.57	6.51
14.5	264	158.96	6.05	869	160.67	5.69
15.0	402	159.03	5.99	1603	161.36	5.85
15.5	406	159.73	6.12	1457	161.37	5.87
16.0	404	160.03	5.98	1256	161.58	5.86
16.5	347	159.83	7.32	1098	161.92	5.93
17.0	307	160.23	6.05	1051	162.04	6.11
17.5	149	160.63	7.36	924	162.03	5.95
18.0	46	161.19	5.58	787	162.00	6.34
18.5	17	160.95	7.25	235	161.17	6.93
Total:	2367			9395		

Fundamentally, therefore, backwardness in regard to somatic development can firstly be experienced in case of the boys trained for skilled work, while in case of the girls this backwardness is of slighter degree and is only manifested in body height.

Our experiences are contradictory to the final conclusions of the studies made in the case of the Budapest boys trained for work in chemical industry, according to

Table 5. The more important parameters of the body height of girls trained for skilled work (n_1) and those attending grammar school and specialised secondary school (n_2), according to half-year age-groups

Age	Girls trained for skilled work			Girls attending grammar school and specialised secondary school		
	n_1	$\bar{x}_1$	s_1	n_2	$\bar{x}_2$	s_2
14.0	25	54.27	11.03	115	51.15	8.53
14.5	264	53.66	9.23	869	53.79	8.20
15.0	402	54.73	9.98	1595	54.39	8.31
15.5	406	54.94	8.78	1453	54.94	8.11
16.0	404	55.01	8.19	1256	55.84	8.34
16.5	347	55.81	9.09	1098	55.99	7.74
17.0	307	56.46	9.13	1051	56.24	7.50
17.5	147	55.63	8.03	924	56.55	7.98
18.0	46	56.57	6.90	787	56.42	8.18
18.5	17	59.40	9.21	235	55.65	7.71
Total:	2365			9383		

Table 6. The more important parameters of the normal chest circumference of girls trained for skilled work (n_1) and those attending grammar school and specialised secondary school (n_2), according to half-year age-groups

Age	Girls trained for skilled work			Girls attending grammar school and specialised secondary school		
	n_1	$\bar{x}_1$	s_1	n_2	$\bar{x}_2$	s_2
14.0	25	85.32	7.76	115	81.96	6.66
14.5	264	84.39	7.77	869	84.26	6.68
15.0	402	85.14	7.77	1603	84.73	6.74
15.5	406	85.59	7.22	1457	85.22	6.66
16.0	404	85.72	6.47	1255	85.95	6.95
16.5	347	86.17	6.96	1098	86.03	6.23
17.0	307	87.14	7.48	1051	86.47	6.26
17.5	149	86.53	6.96	924	86.96	6.56
18.0	46	87.46	5.15	787	86.69	6.61
18.5	17	89.18	7.22	235	86.09	6.49
Total:	2367			9394		

Table 7. Regularity of menses of the girls in the period following the first menstruation

a) Primary school students

Time elapsed since first menstruation (years)	Is the menstruation regular?						Together
	Yes		No		No answer		
	n	%	n	%	n	%	
0	2403	67.0	732	20.4	450	12.6	3585
1	1841	83.2	267	12.0	106	4.8	2214
2	923	86.4	96	9.0	49	4.6	1068
3	248	87.6	24	8.5	11	3.9	283
4	35	85.4	3	7.3	3	7.3	41
5	10	76.9	1	7.7	2	15.4	13
0-5 years	5460	75.8	1123	15.6	621	8.6	7204

b) Secondary school students

0	279	63.0	143	32.3	21	4.7	443
1	918	79.1	219	18.9	23	2.0	1160
2	1956	86.4	271	12.0	36	1.6	2263
3	2160	89.0	220	9.1	47	1.9	2427
4	1631	91.4	136	7.6	17	1.0	1784
5	953	93.8	56	5.5	7	0.7	1016
6	314	94.3	16	4.8	3	0.9	333
7	75	97.4	2	2.6	—	—	77
0-7 years	8286	87.2	1063	11.2	154	1.6	9503

c) Girls trained for skilled work

0	117	66.9	51	29.1	7	4.0	175
1	311	85.7	43	11.8	9	2.5	363
2	606	87.6	60	8.7	26	3.7	692
3	560	87.4	57	8.9	24	3.7	641
4	287	94.1	16	5.2	2	0.7	305
5	132	93.6	9	6.4	—	—	141
6	38	95.0	1	2.5	1	2.5	40
7	11	84.6	—	—	2	15.4	13
0-7 years	2062	87.0	237	10.0	71	3.0	2370

which there are no differences between the somatic development of the Budapest boys attending secondary school and those of receiving vocational training (EIBEN et al. 1979). In our opinion, this may be in connection with the extremely divergent elemental number of the sample.

There is a better conformity, however, between our study results and the experiences of a national survey performed in 9 large cities (BAKONYI, 1984). This author found the means of body measurements of the boys trained for skilled work to be lower in 86.28 p.c.; in case of the girls in 80.40 p.c., as compared to the parameters of the secondary school students of similar age and sex. The latter observation is essential all the more, since it covered several characters (e.g. width measurements, vital capacity, etc.).

2. CHARACTERISTICS OF PUBERTY RELATED TO THE GIRLS

The girls were also questioned in respect to whether their menstruation cycle became regular or not during the time elapsed since their first menstruation (menarche) in both absolute and relative regard. Those cases were regarded as

Table 8. Distribution of discussions within the family about sexual problems related to the girl students trained for skilled work and attending secondary schools

Study site		Type of school						Together			Total
		Inst. for voc. training			Secondary school						
		Yes	No	No answer	Yes	No	No answer	Yes	No	No answer	
County Csongrád	n	908	276	890	2203	563	1376	3111	839	2266	6216
	%	43.8	13.3	42.9	53.2	13.6	33.2	50.0	13.5	36.5	
Trans-danubia	n	63	21	11	566	120	143	629	141	154	924
	%	66.3	22.1	11.6	68.3	14.5	17.2	68.1	15.2	16.7	
Territory east of the river Tisza	n	75	24	39	1690	450	140	1765	474	179	2418
	%	54.3	17.4	28.3	74.1	19.8	6.1	73.0	19.6	7.4	
Northern Hungary	n	33	1	12	1228	196	209	1261	197	221	1679
	%	71.7	2.2	26.1	75.2	12.0	12.8	75.1	11.7	13.2	
Territory between the Danube and Tisza rivers	n	46	11	157	623	219	97	669	230	254	1153
	%	21.5	5.1	73.4	66.4	23.3	10.3	58.0	20.0	22.0	
Together:	n	1125	333	1109	6310	1548	1965	7435	1881	3074	12390
	%	43.8	13.0	43.2	64.2	15.8	20.0	60.0	15.2	24.8	
Total:	n		2567			9823			12390		
	%		20.7			79.3			100.0		

regular menstruation where the cycle was not shorter than 21 days and not longer than 35 days (SAS and KOVÁCS, 1984). Table 7 (a, b, c) comprises the data of students attending primary school, secondary school and those trained for skilled work.

It is to be mentioned in respect to the realistic evaluation of Table 7 that the menarche-median for the studied girls is 12.79 years, the first menstruation appeared at this age in 50 p.c. of the cases. In Hungary this age corresponds to class 7 in primary school. During the first year following the first menstruation, the menses was not regular in a high percentage of the girls (12 — 19 p.c.), which can be regarded as a natural phenomenon. Nevertheless, it is a fact that the cycle was found to be irregular in the 4. — 5 year following the menarche in 5.5 — 7.6 p.c. of the girls attending secondary school and in 5.2 — 6.4 p.c. of the girls trained for skilled work.

A further subject of the analysis was whether the parents discussed questions relating to puberty with the studied students within the family. It was found important to gain a notion about education within the family concerning the correct sexual attitude. The obtained results are summarized in Table 8, according to which 64.2 p.c. of the girls attending secondary school, and 43.8 p.c. of the girls trained for skilled work indicated discussions within the family about the norms of sexual behaviour. This question was left unanswered by the girls trained for skilled work in 43.2 p.c. and by those attending secondary school in 20 p.c. There may be several reasons for this: the girls did not find this question important, they did not understand it, they did not attach particular importance to it, they regarded it a private affair, etc.

Striking differences were experienced analysing the questions according to the geographical regions of Hungary. In Northern Hungary (mainly based on data from county Nógrád) more than 70 p.c. of the girls attending both types of school received information from their parents related to sexual questions. On the contrary, in Southern Hungary (firstly in county Csongrád) only 43.8 p.c. of the girls trained for skilled work and 53.2 p.c. of the girls attending secondary school were able to give positive answers.

Table 9. Distribution of discussions within the family about sexual questions in county Csongrád, according to school types (boys)

Type of school	Yes		No		No answer		Total	
	n	%	n	%	n	%	n	%
Grammar school and spec. second. school	911	49.8	505	27.6	414	22.6	1830	100
Inst. for voc. training	726	44.8	419	25.9	475	29.3	1620	100
Together	1637	47.4	924	26.8	889	25.8	3450	100

Accordingly, well demonstrable deviations can be evidenced between the girls attending secondary school and those receiving vocational training in respect to the degree of sexual education, on the account of the latter.

Simultaneously with our data collection, in county Csongrád a sexual-pedagogical survey was accomplished involving over 7 thousand youths of both sexes receiving some form of sexual education (NÉMETH and GALAMBOS, 1984). The gained experiences confirmed our study results pertaining to discussions within the family about sexual questions (Table 9—10). According to the above mentioned survey — for the availability of which we express our thinks herewith — a fewer number of boys than girls answered "yes" to the aforementioned subject, regarding both types of school.

The experiences from county Csongrád showed that 50 p.c. of the boys already had concrete sexual experiences at the age of 15.39 and the girls at the age of 15.9 (NÉMETH and GALAMBOS, 1984). The problem is on the other hand, that the knowledge of family life and the hygienic culture of the students attending the two types of the school are insufficient.

Discussion

The practical significance of our observations relating to the differences in somatic development first appears at the time of choosing a career. In general, the students trained for skilled work choose physical work, that requires exertion. At the practical occupations they are exerted to physical burden, which even the normally developed young organism is only able to bear after appropriate toughness and accustoming. Since the calcification of the skeleton system is continuous and lasts throughout growth, the child bone may become deformed on the effect of constant burden. The musculature of the youths is also unfit for long-lasting, static burden.

Therefore, at the time of registration for vocational training, the somatic developmental level of the candidates should be inevitably taken into consideration in an increased degree. In case of constitutional deformities it is not correct to choose a trade requiring work carried out in constraint posture (e.g. parquetry).

Table 10. Distributin od discussions within the family about sexual questions in county Csongrád, according to school types (girls)

Type of school	Yes		No		No answer		Total	
	n	%	n	%	n	%	n	%
Grammar school and spec. second. school	1655	69.1	488	20.4	251	10.5	2394	100
Inst. for voc. training	878	69.4	199	15.7	189	14.9	1266	100
Together:	2533	69.2	687	18.8	440	12.0	3660	100

The correct way of living, up-to-date intake of food, proper ratio of active and passive resting are of particular importance at the age of puberty. At the same time, this is the period when the youths start to get accustomed to deleterious habits (smoking, consumption of alcohol and drugs), the chances of which are greater in case of students receiving vocational training as they work in the surroundings of adults.

In the long run, the experiences of our study results point to the insufficiencies of educational work as well, the improvement of which is indispensable in the interest of the adult generation.

References

BAKONYI, F. (1984): A szakmunkás- és középiskolás tanulók testi fejlettségbeli különbségei (Differences in somatic development of students trained for skilled work and those attending secondary school. In Hungarian). — A testn. tanitása. *20*, 5-10.

BUGYI, B. (1965): Beitrag zur Kenntnis der Entwicklung des Muskulatur bei Industrielehrlingen. — Ärztl. Jugendkde. *56*, 267-270.

BUGYI, B. (1966): Methodisches Verfahren zur Ultraschall-Untersuchung der Entwicklung der Muskulatur bei Jugendlichen. — Ärztl.Jugendkde. *57*, 93-97.

BUGYI, B. (1971): Verwandung des Scheider-Curentonschen Testes zur Beurteilung der Leistungsfähigkeit Jugendlicher. — Z. für. ges. Hygiene und ihre Grenzgebiete. *14*, 496-498.

BUGYI, B. (1971a): Egyszerű keringési próbák alkalmazása fiatalokon (Simple circulatory tests in case of youths. In Hungarian). — Testn. Sporteü. Szle. *12*, 163-170.

BUGYI, B. (1971b): Vergleiche einiger Methoden zur Bestimmung des Körperfettes und des Magergewichtes bei Jugendlichen. — Z. f. Ernährungswiss. *10*, 364-381.

BUGYI, B. (1971—1972): Zur Beurteilung des Körperbaus der Industrielehrlinge und der Jungarbeiter in der schweren Industrie in Ungarn. — Glasnik ADJ. *8—9*, 61-63.

BUGYI, B. and LÖRINCZ, L. (1966): A serdülők izomzatának fejlődéséről ultrahang vizsgálatok alapján (On the development of the musculature of teen-agers, based on ultrasound studies. In Hungarian). — Testn. Sporteü. Szle. *5*, 205-214.

EIBEN, O., HEGEDÜS, GY., BÁNHEGYI, M., KISS, K., MONDA, M. and TASNÁDI, I. (1971): Budapesti óvódások és iskolások testi fejlettsége 1968—1969 (Somatic development of kindergartners and school children of Budapest 1968—1969. In Hungarian). — Budapest Fővárosi KÖJÁL kiadása, Budapest. 99 p.

EIBEN, O., KARDOS, I., KOVÁCS, G. and PAPY, I. (1979): Szakmunkástanuló-jelöltek testi fejlettsége, testösszetétele és szomatotipusa (The development, body composition and somatotype of Budapest candidates for vocational training). — Anthrop. Közl. *23*, 53-61.

FARKAS, GY., HUNYA, P., HERENDI, I. and SZEKERES, E. (1983): Studies on the menarcheal age of the girls' of County Csongrád (Southern Hungary). — Acta Biol. Szeged. *29*, 169-178.

GIRINYI, M., KOVÁCS, G., LEZÁK, GY. and NAGY, A. (1977): Szakmunkástanulók, szakközépiskolai tanulók reprezentativ vizsgálata 1976-ban (Representative studies on students trained for skilled work and those attending secondary schools in 1976. In Hungarian). In: KOVÁCS, G. (Ed.): Szakmunkástanulók Országos Egészségvédelmi Intézete Jubileumi Évkönyve 1952—1977. Budapest. 31-93.

SAS, M. and KOVÁCS, L. (Ed.) (1964): Gyermek- és fiatalkori nőgyógyászat (Child- and Juvenile Gynaecology. In Hungarian). — Medicina, Bp. 319 p.

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NEWER PARAMETERS OF THE SOMATIC DEVELOPMENT OF 3—19 YEARS OLD HUNGARIAN CHILDREN AND TEENAGERS

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Abstract

A review is given of the percentile values and graphs used in medical practice, based on the somatic studies performed by the author between the years 1981 and 1984. The reviewed parameters are suitable for controlling the somatic development of youths of appropriate age. The somatic data within the interval ranging from the percentile values of 3 to 97 are indicative of the normal somatic development.
Key words: Hungarian youths, somatic development, standards

Introduction

The controlling of somatic development in the first twenty years following birth is of particular importance. To realize this, however, adequate standards are required.

In general, somatic development is examinde by determining the body height, body weight and normal chest circumference. The somatometric data of a youth can be compared to such tables of physical development which were prepared with the consideration of two parameters — arithmetic mean ($\bar{x}$) and standard deviation (s) — of the corresponding body measures of a child group being of the same age and sex.

In case of the youths of a population, the mentioned tables of somatic development should meet the following requirements:

— The tables of somatic development should be constructed on the basis of the studies of the population to which the youth in question belongs. In such way realistic results cannot be obtained from a comparison between the body measures of the Hungarian youths and the American standards (VÉGHELYI, 1975).

— Since the somatic development of the youths is influences by the social-economical changes, as environmental factors (BERNHOLDT-THOMSEN, 1942; KÁDÁR and VÉLI, 1974; VÉLI, 1948, 1967, 1974), the tables should be checked periodically. Several tables of development were prepared in Hungary previously and these were used throughout the country (EIBEN, 1967; KAROSSA-PFEIFFER and MELLY, 1959;

M. VIOLA, 1952). According to the foregoings these evidently no further meet the requirements.

— When determining the normal values (standards) of somatic development, a method satisfying the practical requirements should be used. This can be obtained by determining either the $\bar{x} \pm 1{,}96.s$ interval, or the appropriate percentile values.

Material and method

The large data collection accomplished between 1981 and 1984 (FARKAS et al. 1983) made it possible to construct a new table of somatic development.

The body weight, body height and normal chest circumference of the youths were determined according to the method of MARTIN (MARTIN and SALLER, 1956). The age was defined by R-55 type computer using the decimal table (FARKAS et al. 1983) and the parameters were calculated according to half-year age-groups.

Tables containing the 3, 25, 50, 75 and 97 percentiles were prepared for the three body measures (Table 1), based on which the growth graphs were also drawn (Figs. 1. — 6.).

Discussion

Due to the fact that the reviewed tables and graphs concern about 50 thousand youths, who — even if not in even distribution — live at the most varied settlements of Hungary, we find our data — especially those pertaining to the youths between the age 10 — 18 — characteristic to the Hungarian children.

The measurements were taken by the same person with the same instruments throughout the whole period of the data collection. Accordingly, the deviations between the arithmetic means of the age-groups and sexes cannot be explained by various techniques. The abnormal deviations occurring at the lowest and highest age-groups (the mean of youths belonging to the older age-groups is lower as compared to the younger ones) can be interpreted by the so-called cross-sectional study character of the data collection. Taking every aspect into consideration — according to our judgement — the reported tables and graphs of development meet the afore-mentioned requirements, thus they are recommended for use in case of the Hungarian children. At the same time, in case of the youths of Hungary as well as other populations being of identical age and sex, the somatic development becomes comparable with the help of the calculated parameters.

Table 1. Percentiles of body height, body weight and normal chest circumference between the ages of 3—19

Percentiles (boys)					Age (years)	Percentiles (girls)				
3	25	50	75	97		3	25	50	75	97
					3					
91.2	95.7	98.1	100.5	105.1	cm	89.8	94.4	97.0	99.5	104.3
12.8	14.7	15.7	16.7	18.7	kg	11.2	14.0	15.5	17.0	19.8
48.7	51.4	52.9	54.3	57.1	cm	46.4	50.2	52.3	54.4	58.3
					3.5					
91.4	96.7	99.6	102.4	107.8	cm	91.3	96.0	98.5	101.0	105.7
12.5	14.6	15.8	16.9	19.0	kg	12.2	14.3	15.4	16.5	18.7
48.5	51.1	52.6	54.0	56.6	cm	47.1	50.0	51.5	53.0	56.0
					4					
95.6	100.9	103.8	106.6	112.0	cm	95.9	100.7	103.4	105.9	110.8
13.0	15.9	17.5	19.0	22.0	kg	12.5	15.4	17.0	18.5	21.5
48.8	52.2	54.1	55.9	59.4	cm	47.3	50.7	52.6	54.4	58.0
					4.5					
94.0	102.5	107.2	111.7	120.5	cm	98.4	103.1	105.8	108.2	113.1
14.2	16.8	18.3	19.7	22.4	kg	13.1	16.0	17.6	19.2	22.2
50.0	53.0	54.7	56.3	59.4	cm	48.0	51.5	53.3	55.1	58.6
					5					
100.2	106.6	110.1	113.5	120.0	cm	101.3	106.8	109.8	112.7	118.4
13.7	17.2	19.1	21.0	24.5	kg	13.6	17.1	19.1	20.9	24.5
49.7	53.4	55.5	57.4	61.2	cm	48.7	52.5	54.6	56.6	60.5
					5.5					
104.1	109.5	112.5	115.3	120.9	cm	104.6	110.1	113.2	116.1	121.8
15.2	18.2	19.9	21.5	24.7	kg	14.5	18.3	20.3	22.3	26.2
51.1	54.2	55.9	57.6	60.8	cm	48.8	53.2	55.6	57.9	62.4
					6					
108.6	113.6	116.4	119.1	124.3	cm	105.9	112.3	115.8	119.1	125.6
16.0	19.5	21.4	23.2	26.7	kg	15.0	19.0	21.3	23.5	27.6
51.5	55.0	57.0	58.9	62.5	cm	49.6	54.0	56.3	58.6	63.1
					6.5					
108.5	114.5	117.8	121.0	127.2	cm	107.6	131.8	117.3	120.6	127.0
16.3	19.9	21.9	23.8	27.5	kg	12.9	18.3	21.2	24.1	29.6
51.8	55.5	57.5	59.5	63.3	cm	49.4	54.0	56.6	59.1	63.9
					7					
110.4	117.4	121.3	125.0	132.2	cm	110.1	117.1	121.0	124.7	131.8
15.8	20.9	23.7	26.3	31.5	kg	17.2	21.0	23.1	25.1	29.0
51.3	56.2	59.0	61.6	66.6	cm	51.0	55.3	57.7	60.0	64.4
					7.5					
114.5	121.0	124.7	128.1	134.8	cm	114.0	120.2	123.6	126.9	133.2
17.8	22.2	24.7	27.0	31.6	kg	16.6	21.7	24.5	27.2	32.4
53.5	57.5	59.6	61.7	65.7	cm	51.2	56.1	58.7	61.3	66.3
					8					
117.6	123.9	127.3	130.6	137.0	cm	116.5	122.7	126.2	129.5	135.8
18.4	23.5	26.3	29.0	34.3	kg	17.0	22.4	25.4	28.3	33.9
53.7	58.6	61.3	63.8	68.8	cm	51.3	56.7	59.6	62.4	67.7

Table 1. (Continuation 1.)

Percentiles (boys)					Age (years)	Percentiles (girls)				
3	25	50	75	97		3	25	50	75	97
					8.5					
118.9	126.1	130.1	133.9	141.3	cm	117.2	124.9	129.1	133.2	141.0
19.4	24.8	27.8	30.7	36.2	kg	17.9	23.8	27.1	30.3	36.4
55.0	59.8	62.4	65.0	69.9	cm	52.4	58.0	61.1	64.0	69.7
					9					
120.7	128.0	132.0	135.9	143.4	cm	119.0	127.3	132.0	136.4	145.0
19.2	25.3	28.7	31.9	38.2	kg	17.7	24.7	28.6	32.3	39.6
54.4	60.1	63.2	66.2	72.0	cm	51.9	58.5	62.2	65.7	72.4
					9.5					
122.6	130.5	134.9	139.0	147.1	cm	122.3	130.4	134.8	139.1	147.3
20.3	27.0	30.8	34.3	41.2	kg	17.8	26.3	30.9	35.4	44.1
55.0	61.0	64.4	67.5	73.7	cm	52.0	59.9	64.2	68.4	76.5
					10					
126.5	133.9	138.0	142.0	149.6	cm	125.9	134.2	138.8	143.2	151.7
20.8	28.6	32.9	37.0	45.0	kg	18.9	28.1	33.3	38.1	47.5
55.8	62.3	65.9	69.4	76.0	cm	52.6	60.9	65.5	69.9	78.4
					10.5					
129.4	137.0	141.1	145.2	152.9	cm	128.8	137.0	141.5	145.9	154.3
21.3	30.1	34.9	39.5	48.4	kg	21.2	30.3	35.3	40.1	49.4
56.1	63.1	67.0	70.7	77.9	cm	54.2	62.6	67.2	71.6	80.2
					11					
130.8	138.7	143.1	147.3	155.4	cm	131.2	139.7	144.5	149.0	157.7
22.4	31.4	36.3	41.1	50.3	kg	21.9	31.8	37.3	42.5	52.7
57.0	64.3	68.3	72.2	79.7	cm	55.1	64.1	69.0	73.8	82.9
					11.5					
133.0	141.2	145.7	150.1	158.5	cm	133.5	142.4	147.3	152.0	161.2
23.0	32.8	38.3	43.4	53.5	kg	22.6	33.5	39.6	45.5	56.7
57.7	65.3	69.6	73.7	81.5	cm	55.9	65.7	71.2	76.5	86.6
					12					
134.6	143.6	148.6	153.3	162.5	cm	137.0	145.8	150.7	155.3	164.3
24.1	34.7	40.5	46.1	56.9	kg	25.2	36.4	42.6	48.5	60.0
58.5	66.7	71.2	75.5	83.9	cm	58.7	68.5	73.9	79.0	89.0
					12.5					
136.4	145.9	151.2	156.3	166.1	cm	140.5	148.9	153.5	157.9	166.6
24.7	36.2	42.5	48.6	60.3	kg	27.2	38.9	45.4	51.6	63.6
59.4	67.9	72.6	77.1	85.9	cm	61.0	71.1	76.7	82.0	92.3
					13					
139.5	149.5	155.1	160.4	170.8	cm	143.1	151.3	155.8	160.1	168.5
26.8	39.0	45.7	52.2	64.7	kg	30.0	41.0	47.0	52.8	64.0
61.3	70.0	74.8	79.4	88.2	cm	63.5	72.9	78.2	83.2	92.8
					13.5					
142.4	152.7	158.4	163.9	174.4	cm	145.5	153.4	157.8	161.9	170.0
28.5	41.1	48.2	54.9	67.9	kg	31.4	43.2	49.7	56.0	68.0
62.5	71.4	76.4	81.1	90.2	cm	65.2	75.2	80.7	85.9	96.1

Table 1. (Continuation 2.)

Percentiles (boys)					Age (years)	Percentiles (girls)				
3	25	50	75	97		3	25	50	75	97
					14					
146.0	156.4	162.2	167.7	178.4	cm	147.5	155.0	159.1	163.1	170.7
31.2	44.5	51.9	58.9	72.6	kg	34.3	45.1	51.1	56.8	67.9
64.8	74.0	79.0	83.9	93.3	cm	67.9	76.8	81.8	86.5	95.7
					14.5					
149.0	159.3	165.1	170.6	181.2	cm	148.5	155.7	160.0	163.6	171.1
33.9	47.0	54.3	61.2	74.6	kg	36.0	47.0	53.2	59.0	70.3
67.3	76.1	81.0	85.7	94.7	cm	69.7	78.8	83.9	88.7	98.1
					15					
152.8	162.5	167.8	172.9	182.9	cm	149.4	156.7	160.8	164.6	172.1
37.2	50.0	57.1	63.9	77.0	kg	37.9	48.5	54.3	59.9	70.8
69.9	78.4	83.1	87.6	96.4	cm	71.6	80.1	84.7	89.2	97.8
					15.5					
156.8	165.6	170.5	175.2	184.2	cm	149.7	157.0	161.0	164.8	172.2
39.8	52.6	59.7	66.4	79.5	kg	39.3	49.3	54.9	60.2	70.5
72.2	80.5	85.1	89.5	98.1	cm	72.5	80.7	85.3	89.6	98.1
					16					
158.4	166.9	171.6	176.2	184.9	cm	150.0	157.2	161.1	164.9	172.3
42.6	54.8	61.6	68.0	80.5	kg	39.9	50.0	55.6	60.9	71.2
74.2	82.1	86.5	90.7	98.8	cm	73.0	81.3	85.8	90.2	98.7
					16.5					
160.7	168.8	173.2	177.5	185.7	cm	149.4	157.1	161.4	165.5	173.4
43.5	56.7	64.1	71.1	84.6	kg	40.7	50.5	55.9	61.1	71.2
74.7	83.4	88.3	92.9	101.8	cm	74.0	81.8	86.1	90.2	98.1
					17					
160.9	169.0	173.4	177.7	186.0	cm	150.1	157.5	161.6	165.6	173.2
45.9	58.1	64.9	71.4	84.0	kg	41.4	51.0	56.3	61.3	71.1
76.7	84.7	89.1	93.3	101.5	cm	74.3	82.2	86.6	90.8	98.9
					17.5					
162.2	170.1	174.4	178.5	186.6	cm	150.2	157.7	161.8	165.8	173.5
47.3	59.2	65.7	72.0	84.1	kg	41.4	51.1	56.4	61.6	71.5
77.6	85.3	89.6	93.6	101.5	cm	74.5	82.5	86.9	91.2	99.4
					18					
162.6	170.9	175.5	179.9	188.4	cm	151.1	158.1	162.0	165.8	173.0
49.3	60.9	67.3	73.5	85.4	kg	41.2	51.0	56.4	61.6	71.7
79.2	86.3	90.2	93.9	101.2	cm	74.5	82.4	86.7	90.9	99.0
					18.5					
162.1	170.1	174.6	178.8	187.1	cm	149.5	157.1	161.4	165.4	173.3
48.9	59.7	65.6	75.3	82.4	kg	40.9	50.5	55.5	60.9	70.7
79.2	86.3	90.3	94.0	101.3	cm	74.0	81.9	86.3	90.5	98.7
					19					
159.6	167.7	172.2	176.5	184.8	cm	150.1	157.2	161.1	164.8	172.1
48.6	61.7	68.9	75.9	89.3	kg	41.3	51.7	57.5	63.0	73.7
80.0	88.7	93.6	98.2	107.1	cm	75.0	82.8	87.1	91.2	99.1

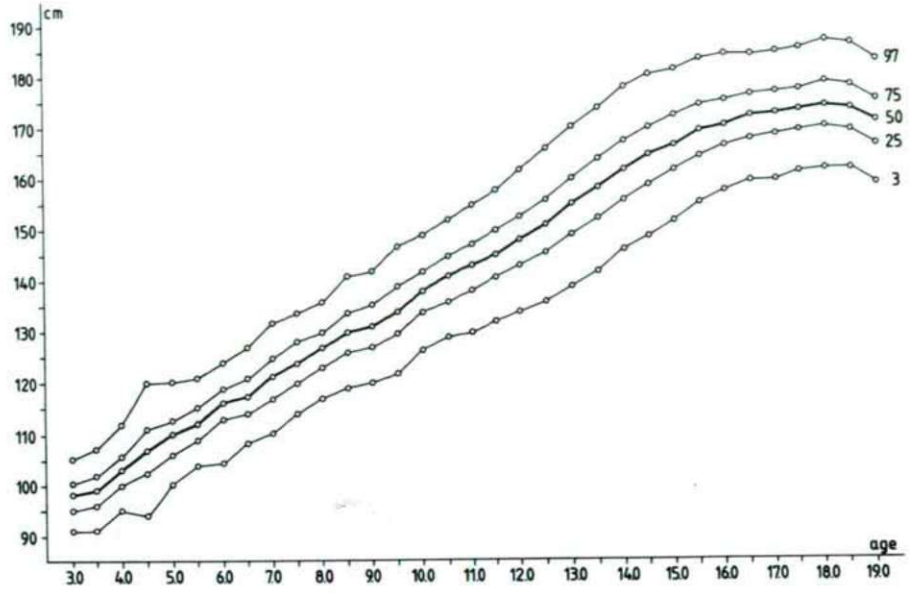

Fig. 1. Percentile curves of the stature of boys between the ages of 3 — 19

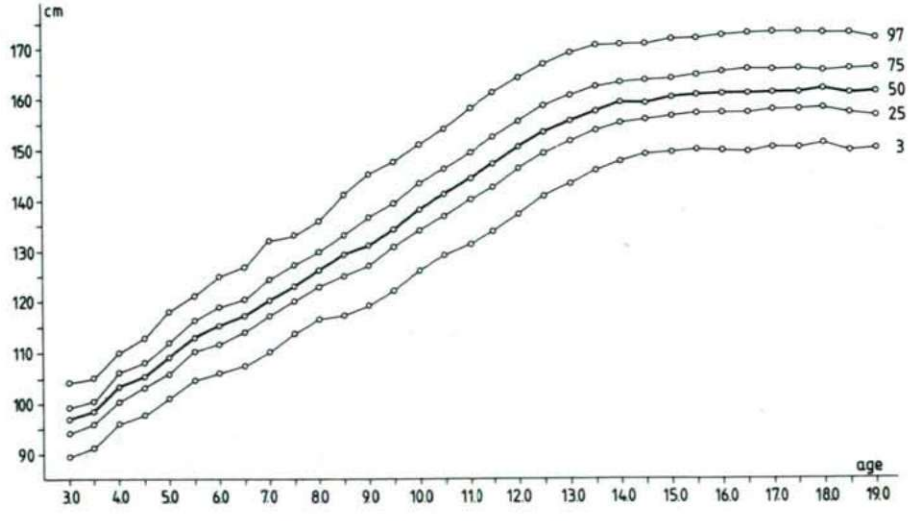

Fig. 2. Percentile curves of the stature of girls between the ages of 3 — 19

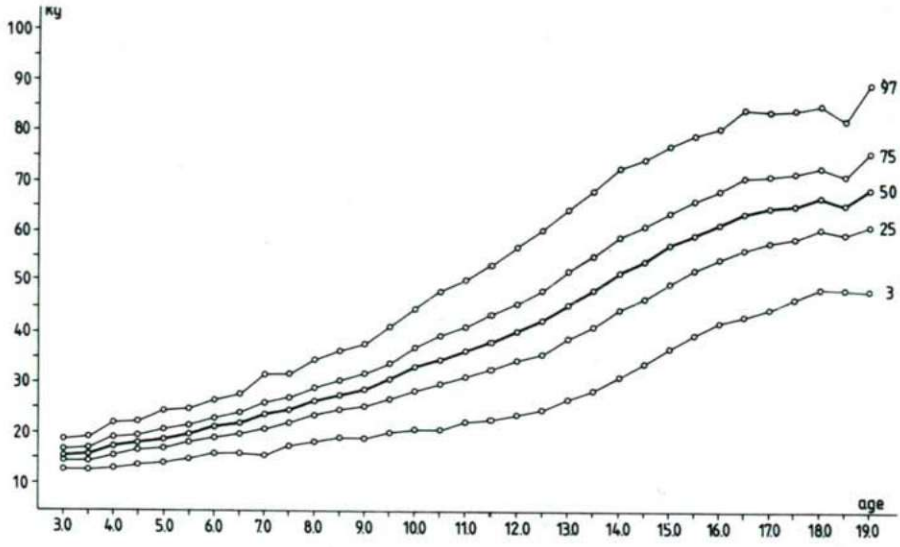

Fig. 3. Percentile curves of the body weight of boys between the ages of 3—19

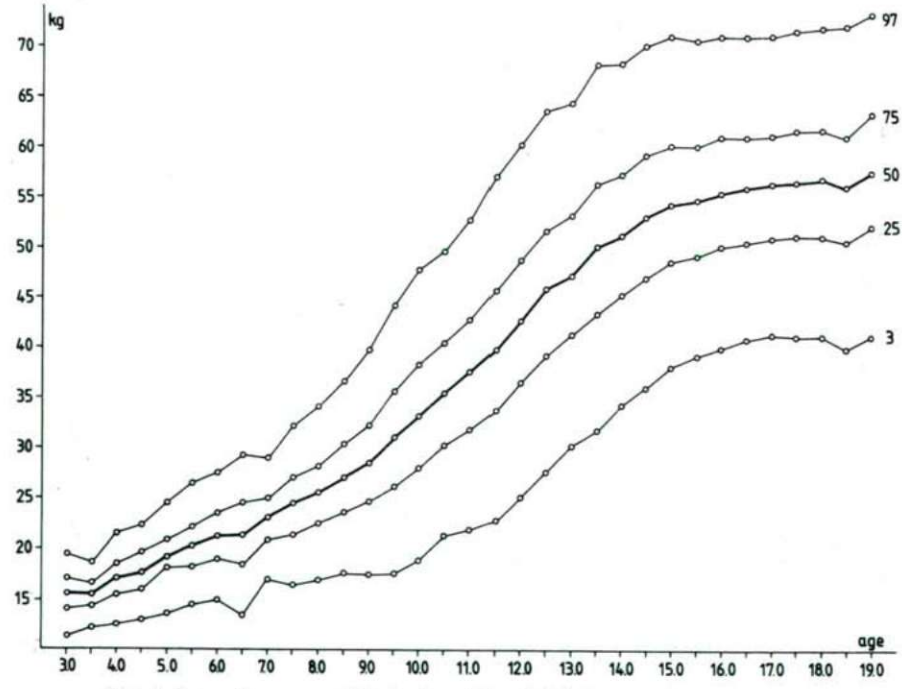

Fig. 4. Percentile curves of the body weight of girls between the ages of 3—19

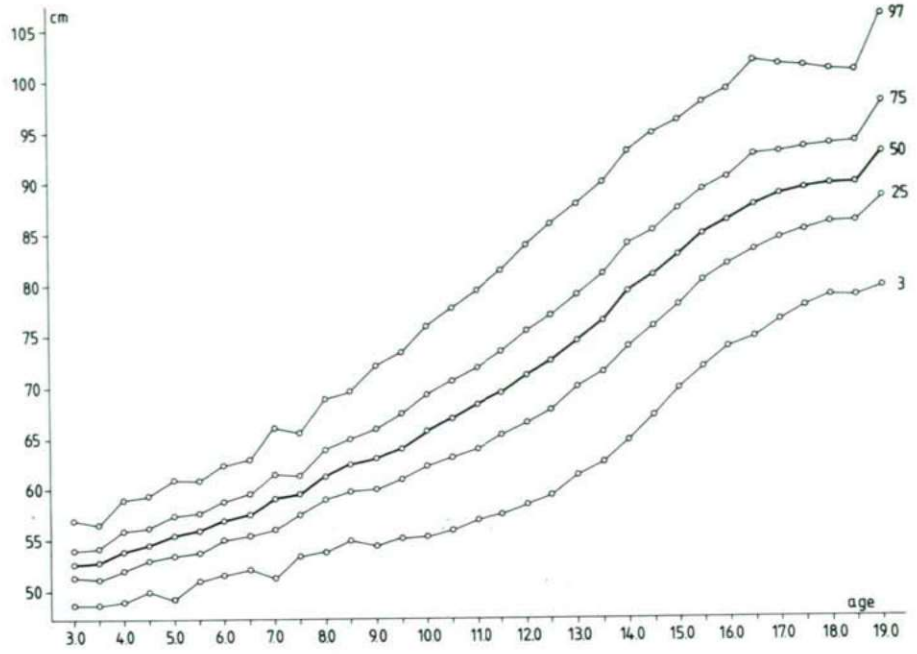

Fig. 5. Percentile curves of the normal chest circumference of boys between the ages of 3—19

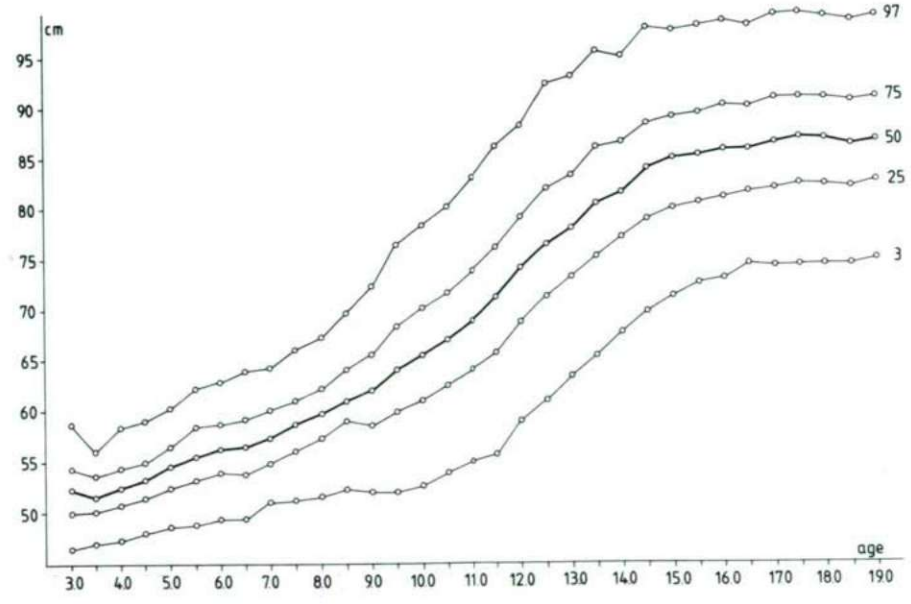

Fig. 6. Percentile curves of the normal chest circumference of girls between the ages of 3—19

References

BENNHOLDT-THOMSEN, C. (1942): Die Entwicklungsbeschleunigung der Jugend. — Ergeb. inn. Medizin. *62*, 1153.

EIBEN, O. (1967): A pubertáskor anthropológiai problémái (Anthropologische Probleme der Pubertät). — Gyermekgyógyászat. *18*, 453-457.

FARKAS, GY., HUNYA P., HERENDI, I. and SZEKERES, E. (1983): Studies on the menarcheal age of the girls of county Csongrád (Southern Hungary). — Acta Biol. Szeged. *29*, 169-178.

KAROSSA-PFEIFFER, J. and MELLY, J. (1955): Az iskolaorvos zsebkönyve (Vade-mecum of medical superintendent). Budapest.

KÁDÁR, P. and VÉLI, GY. (1974): Az akceleráció szakaszosságáról (On the periodicity of acceleration). — Anthrop. Közl. *18*, 105-111.

VÉGHELYI, P. (1975): Gyermekgyógyászati vademecum (Pediatrical vade-mecum). Budapest.

VÉLI, GY. (1948): Mennyire befolyásolta a háború a gyermekek testi fejlődését? (The Influence of the War on the Physical Development of the Children). — Népegészségügy. *29*, 669.

VÉLI, GY. (1967): Az akceleráció a felszabadulás előtt és után (Die Acceleration vor und nach der Befreiung). — Anthrop. Közl. *11*, 25-30.

VÉLI, GY. (1974): Akceleráció-e a säcularis trend? (Bedeutet der säkulare Trend eine Akzeleration? — Gyermekgyógyászat. *25*, 420-422.

M.VIOLA, I. (1952): Fejlődési táblázat. Budapest.

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PROGNOSTIC METHOD FOR THE DETERMINATION OF THE MENARCHE-AGE

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Abstract

A report is given of a procedure by which the time of the first menstruation (menarche) can be prognosticated with the help of the so-called predictive additive method. Such factors are taken into account by the method that are easily recognizable in primary school practice. The procedure is considered valid in case of the Hungarian girls, nevertheless on its basis, a similar prognostic possibility can be elaborated in respect to other ethnic groups.
Key words: prognosis of menarche, predictive additive method

Introduction

One of the most authentic indicators of the puberty of girls is the first menstruation, or menarche. This phenomenon has been studied in numerous countries from several viewpoints. However, no example can be found in the international literature in respect to the accomplishment of a simultaneous multiple data collection in the case of a high number of girls belonging to the same population.

This recognition prompted us to organise a large-scale data collection in Hungary in 1981 to attempt the characterization of the relationships detectable between partly the biological, partly the social factors and the menarche (FARKAS et al. 1983). The partial results have already been reported on several occassions (FARKAS, 1982, 1985; FARKAS et al. 1983, 1983a, 1985; FARKAS and NAGY, 1981, FARKAS and SZEKERES, 1982, 1982a; FARKAS and TAKÁCS, 1986) and are presently under publication, resp. (FARKAS, in press 1, 2; FAZEKAS et al. 1983).

Our aim was to provide concrete help to the sexual-hygienic educational work in primary schools.

Material and method

The data collected by means of questionnaires were evaluated by R-55 type computer following uniform coding. Since our sample is based on the data of girls living at various regions of the country — but firstly in county Csongrád — it is found suitable for making generalization on its basis in regard to the Hungarian girls. Our sample involves approximately 30 thousand girls.

As the final outcome of the evaluation, a so-called additive predictive model was elaborated with the help of which it is possible to prognosticate, the age at with the first menstruation takes place in case of a given girl.

The basis of the method is as follows:

Relationship can be concluded between the time of the first menstruation of the girls and the factors studied by us (e.g. the size of the settlement where the girl lives, her order of birth, number of sibs, school achievements, occupation and educational level of parents, etc.). The connection can be characterized numerically; namely, if it promotes puberty, this correlation value is negative, if it retards, the value is positive.

Table 1. Coefficients applicable for the estimation of the absolute menarche-age according to the girl's domicile, year of birth and the father's occupation

Girl's county of domicile	Coefficient
Baranya	0.036
Bács-Kiskun	–0.076
Békés	0.075
Borsod-Abaúj-Zemplén	0.029
Csongrád	–0.008
Heves	–0.156
Komárom	–0.110
Nógrád	0.261
Pest	0.172
Somogy	–0.081
Szabolcs	0.006
Szolnok	–0.213

Girl's year of birth	Coefficient
1963	0.623
1964	0.495
1965	0.429
1966	0.287
1967	0.155
1968	–0.039
1969	–0.274
1970	–0.708
1971	–1.222
1972	–1.808

Occupation of father	Coefficient
Industrial manual worker	0.030
Agricultural manual worker	0.044
Manual worker in other sphere	0.012
Intellectual (education of higher grade)	–0.026
Intellectual (secondary education)	0.016
Pensioner	0.044
Deceased	0.056
Other	0.092

On the other hand, in knowledge of the precise data of a girl's birth and her first menstruation, her absolute menarche-age can be calculated, i.e. the difference between her dates of birth and menarche. This age was determined in the case of approximately 20 thousand girls and since the calculation was performed on the basis of the decimal table (FARKAS et al., 1983), its arithmetic mean was also determinable. This is called the main-average of menarche of the population, which is 12.534 years in case of our sample.

If we wish to estimate the probable time of the first menstruation in case of a girl not menstruating yet, this can be achieved by the following formula, with the application of the predictor coefficients shown in Tables 1.—3.:

$y = \bar{y}$ + the coefficients of the appropriate predictors, where y = the estimated menarche-age, $\bar{y}$ = the main-average of menarche of the population.

Results

Let us assume that a girl's domicile is county Csongrád, she was born in 1967, her father is an agricultural manual worker, her mother is an industrial manual worker, the mother was 25 years old when her girl was born, the girl's hair-colour is

Table 2. Coefficients applicable for the estimation of the absolute menarche-age according to mother's occupation, age and the girl's hair colour

Occupation of mother	Coefficient
Industrial manual worker	0.030
Agricultural manual worker	–0.022
Manual worker in other sphere	0.027
Intellectual (education of higher grade)	0.027
Intellectual (secondary education)	–0.025
Pensioner	0.051
Deceased	–0.051
Housewife	0.109

Mother's age at time of girl's birth years	Coefficient
15–19 years	–0.058
20–24 years	0.021
25–29 years	0.068
30–34 years	0.042
35–39 years	–0.030
40–44 years	–0.012
45–49 years	0.077

Girl's hair colour	Coefficient
Light blonde	–0.156
Blonde	0.052
Dark blonde	0.013
Brown	0.025
Dark brown—black	0.010
Red	–0.079

brown, 69 air kilometres is the distance between the place of birth of the parents, the size of the domicile of the girl is under 5 thousand inhabitants, she has two living sisters and one living brother, and she was born fourth. Using the above formula as the appropriate coefficients of Tables 1.—3., her menarche-age would be the following: $y = 12.534 - 0.008 + 0.155 + 0.044 + 0.030 + 0.068 + 0.025 + 0.046 - 0.083 + 0.114 + 0.024 - 0.024 = 12.925$ years.

According to our estimation the first menstruation of the girl in question should have taken place at the age of 12.925. Our example is related to a concrete girl, who was born on 6th November, 1967 and the date of her first menstruation was 9th January, 1981. On the basis of the decimal table, her absolute menarche-age was at 13.175 years, i.e. 0.25 years — quasi four months — later than estimated by means of our method.

Studying the formula in the case of 100, absolutely randomly selected, already menstruating girls, a deviation of 0.2546 years was found between the empiric and estimated menarche-age, i.e. the occurrence of the menarche was averagely estimated later.

The individual comparison of the empiric and estimated menarche-age showed deviation between the two values mainly in the case of girls reaching maturity at very early or very late age. With the method, besides 95% probability level, 51% of the real menarche time fell below the confidence interval of the estimated menarche time (estimation to earlier date), 34% fell into the interval and 15% above the upper limit value (estimation to a later date). This means that with the method, with 95% probability, the menarche-age can be estimated to an earlier, or to the same time in 85%, and to a later time in 15% of the cases as compared to the actual occurrence of the menarche-age.

Discussion

It might seem as if the method can only be applied with fairly great error. It is a fact, however, that the genetically defined time of the menarche may considerably be modified by environmental factors; among these, mainly by the social ones, and this also influences the accuracy of our estimation.

The demonstration of the method did not have the purpose of giving an infallible solution to the estimation of the menarche-age, since based on the above mentioned things it seems to be impossible — at least for the time being. Our primary aim was to demonstrate that in the possession of appropriate information the opportunity presents itself to obtain a fairly exact approach to the time of occurrence of the menarche, as an indicator of puberty. Nevertheless, it should be emphasized that the application of this model in cases of another (not Hungarian) population is possible only if the correlation values characteristic to the given population are at disposal, based on similar surveying.

Table 3. Coefficients applicable for the estimation of the absolute menarche-age according to the air-distance of the place of birth of parents, size of girl's domicile, number of living brothers and sisters, and order of birth

Air-distance of parent's place of birth	Coefficient
0 km	0.024
1— 25 km	0.015
26— 50 km	0.023
51—100 km	0.046
101—200 km	–0.023
201—440 km	0.040

Size of girl's domicile	Coefficient
100.000—200.000	–0.320
50.000—100.000	–0.077
10.000— 50.000	–0.106
5.000— 10.000	–0.071
below 5000	–0.083

Number of living sisters	Coefficient	Number of living brothers	Coefficient
0	–0.032	0	–0.048
1	0.049	1	0.024
2	0.114	2	0.051
3	0.098	3	0.104
4	0.213	4	–0.001

Girl's order of birth	Coefficient
1	–0.006
2	0.064
3	–0.036
4	–0.024

References

FARKAS, GY. (1982): On social and natural factors exerting influence on maturation of girls. — Anthrop. Közl. *26*, 135-139.

FARKAS, GY. (1985): A leányok serdülését befolyásoló tényezők ismeretének jelentősége a szexuális nevelésben (Significance of knowledge on the factors influencing the puberty of girls in sexual education. In Hungarian.) — Módszertani Közl. *25*, 227-235.

FARKAS, GY. (in the press 1.): Die Grösse des Menarche-Medians und die Zusammenhänge mit endogenen und exogenen Faktoren in Ungarn. — Ärztl. Jugendkde.

FARKAS, GY. (in the press 2.): Relationship between the different factors and menarche-age in Hungary. — Anthrop. Közl.

FARKAS, GY., FAZEKAS, A. and SZEKERES, E. (1983): The fluoride content of drinking water and the menarcheal age. — Acta Biol. Szeged. *29*, 159-167.

FARKAS, GY., HUNYA, P., HERENDI I. and SZEKERES, E. (1983a): Studies on the menarcheal age of the girls of county Csongrád (Southern Hungary). — Acta Biol. Szeged. *29*, 169-178.

FARKAS, GY., HUNYA, P. and SZEKERES, E. (1985): Relationship of the menarcheal age of city girls with socio-economic factors. — Acta Biol. Szeged. *31*, 183-190.

FARKAS, GY. and NAGY, J. (1981): Über das Menarchealter von Mädchen aus Komárom. — Acta Biol. Szeged. *27*, 223-227.

FARKAS, GY. and SZEKERES, E. (1982): On the puberty of girls in Szeged, Hungary. — Acta Biol. Szeged. *28*, 155-165.

FARKAS, GY. and SZEKERES, E. (1982a): Eine vergleichende Untersuchung der körperlichen Entwicklung und der körperlichen Reifung von Mädchen aus Szeged (Ungarn). — Humanbiol. Budapest. *12*, 237-243.

FARKAS, GY. and TAKÁCS, T. (1986): Comparison of somatic characters in menstruating and not menstruating girls. — Acta Biol. Szeged. *32*, 183-189.

FAZEKAS, A., FARKAS, GY., SZEKERES, E. and KOCSIS, S.G. (1983): A gyermekek testi fejlettsége különböző fluorid koncentrációjú ivóvizű településeken (The somatic development of children consuming drinkwater with different fluoride concentrations in two Hungarian settlements). — Anthrop. Közl. *27*, 119-127.

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THESIS OF DISSERTATIONS FOR CANDIDATE DEGREES

HORMONAL REGULATION OF THE DORMANCY AND GERMINATION OF SEEDS REQUIRING STRATIFICATION*

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Introduction

In the course of the few decades since the discovery of plant hormones, we have become familiar with several aspects of growth regulation, nevertheless, the regulation of the cessation in growth — the dormancy — is still unclear even nowadays.

In respect to the physiology of dormancy, the assumption is generally accepted that its development, maintenance and release are regulated by hormonal balance. As to the hypothesis regarding regulation by hormonal balance, the question arises what changes are brought forth by environmental factors in the metabolism and interaction of growth-regulating substances? Studies on the latter problem are of particular importance in case of the seeds manifesting deep dormancy which require special environmental conditions for dormancy release, for germination. From practical point of view, the most noteworthy from the special environmental requirements is low temperature.

A long known forestry experience is that most of the deciduous tree seeds of the forest do not germinate in the first spring following autumn sowing after harvest. During the course of adaptability to the environment, such a degree of inhibition develops in these seeds that only ceases on the effect of cold. This is when activation starts, ensuring the germination of these seeds in spring, after the cold period — the natural winter cold. However, the seed is only capable of perceiving the cold effect in swollen state; and the swelling is a rather slow process taking months owing to the presence of the fruit- and seed-coats.

By means of treatments substituting the natural effects, as well as studies in connection with the physiological role of stratification, numerous valuable data have been obtained during the past few years. Despite these, however, it cannot be said that the physiological processes taking place at low temperature have been clarified. It is assumable that many common features exist in the mechanism of the

* *Based on a dissertation for candidate degree defended on November 9th, 1984.*

warming up phases in case of different seeds requiring stratification, but the data at hand do not make any generalizations possible.

Due to the lack and fractional character, resp., of the comparative studies (hormonal changes taking place in seeds being in deeply dormant and not deeply dormant state; changes taking place during cold- and warm-stratification, resp.), at present no essential differences can be concluded between the hormonal regulation of the germination of seeds with ordinary and special environmental demands, resp., furthermore, it cannot be revealed which are the physiological changes taking place only in case of low-temperature treatment.

Our studies aimed at investigating the role of gibberellin (GA) from germination-physiological point of view. The major questions which we wished to receive answer in our experiments were the followings:

— Is the breaking of dormancy and the start of germination in connection with the appearance of the endogenous free GA-s?
— Are there any differences in tendency in the changes of the endogenous GA-level during the preparation for germination of the seeds being in deeply dormant and those of in not deeply dormant state?
— What are the changes taking place in the endogenous GA-level of the seeds being in deeply dormant state under environmental conditions unfavourable for germination?
— What is the role of bound gibberellin in the changes of the endogenous GA-level in case of seeds being in deeply dormant state?
— Are there any essential differences in the endogenous gibberellin-spectrum of the different seeds?
— Does de novo GA synthesis have any significance in the early phase of germination?
— What is the possible role of abscisic acid (ABA) in the regulation of the endogenous GA-level?
— Which are the hormonal ratios and interactions regulating dormancy and its cessation?

To determine whether there are any differences between the hormonal changes taking place at the time of germination induced under special conditions and the hormonal changes in the seeds not requiring any special treatment for germination, comparative studies have been performed in accordance with the afore-listed viewpoints. Since from theoretical and practical points of view there are no literary data available on the germination-physiology of our most important study material — the linden seed —, the dissertation comprises the general physiological characterization of the dormant linden-seeds.

Materials and methods

Tilia sp., *Fraxinus excelsior*, *Phaseolus* sp. and *Lupinus albus* seeds were used in our comparative studies. The procumbent period of the deeply dormant, hard-coated seeds was shortened by means of

chemical scarification, performed after the fertilization of the seeds in heated sand moistened to 70 % of the total water capacity, at 25 and 4 — 6 °C, resp.

The methods of extraction and diffusion were used to study the changes in endogenous gibberellin-content. The gibberellins in the extract and diffusate, resp., were divided into fractions in dissolvents, according to their pH-dependent differing distribution, then were separated further by thin-layer chromatography. The biological activity measurements and the pertaining dosimetry were performed with barley endosperm and lettuce hypocotyl tests. The GA chromatogram-spots were identified on the bases of the Rf value measured on different adsorbents and several solvent-systems, the GA_3-related movement, induced fluorescence of the spots, colour-reactions, behaviour in various biological tests, as well as IV-spectrum; using authentic compounds.

The exogenous ABA content and the CCC amount absorbed by the seeds were determined with UV-spectrophotometry following thin-layer-chromatography, and Dragendorff reaction, resp. The possible interactions between the different hormones (GA, IAA, KIN, ABA) were modelled in embryo culture.

The leaf-pigments were measured by photometry, the protein content was determined according to Lowry et al. To follow the changes in the level of the growth inhibitors, straight-growth coleoptyl-tests, several germinating tests and linden embryo tests were used. The ABA-related instrumental measurement results were complemented with stoma-closing test, the GA-related ones with dwarf-pea and dwarf-maize tests. Several histological techniques were also applied in the course of the studies.

The mechanical role of the endosperm as well as the correlation effects between the different parts of the embryo were studied by means of operative interventions. Cellulase- and pectinase-activity measurements were made when studying the interaction between the embryo and the endosperm. The nuclear DNA-content of the embryonic cells was measured by cytophotometry following Feulgen-staining, the DNA + RNA contents following GCA-staining.

Summary of the new scientific results

The dormancy type of the *Tilia platyphyllos* seeds as well as the basic processes leading to the cessation of dormancy were clarified.

It was determined that the linden seeds represent the type of morpho-physiological dormancy state where the fruit- and seed-coats do not have any decisive role, but the cotyledone and endosperm play an active regulatory role; and the cold effect cannot be substituted by GA. The cold-stratification is necessary for the induction of the conditions ensuring harmonious embryo-growth as well as for launching the synthesis of the hydrolases responsible for the cytolysis of the part of the endosperm around the radicle. Accordingly, we have characterized a special type of the seed dormancy, which has not been described yet.

We have demonstrated that a certain amount of the GA-s is in free form in booth the deeply dormant and non-deeply dormant type dry seeds. Thus, from the viewpoint of germination, not the appearance of the free gibberellins is the decisive motif.

In the course of preparations for germination we have experienced the elevation of the endogenous free GA-level in both seed types. Therefore, the free GA-s should be present in a determined quantity in a determined phase of the swelling process for the biochemical and physiological processes of germination, furthermore, for ensuring normal growth of the seedlings. The change in GA-level according to the maximum-curve is a general observation in the early phase of

germination. The time-point of the development of the GA-maximum, however, is not identical in case of the seeds in deeply dormant state.

The differences manifesting in the sequence of the maxima of the promotor hormones during cold-stratification can be correlated with the success of the exogenous hormone-treatments. Since in the *Tilia* seeds the GA-maximum develops only at the end of the cold-stratification, exogenous GA-treatment applied during warm-stratification is not effective in breaking of dormancy

The raise in the endogenous GA-level is measurable under non-inductive circumstances, too (during warm-stratification), it also indicates the fact that elevation of the GA-level in itself is not sufficient for stopping the dormant state.

It has been demonstrated that the raise in the amount of free GA-s is a process parallel with the decrease in the amount of bound GA-s. The genetic relationship between the free and bound GA-s is also referred to by the fact that upon the hydrolysis of the bound fraction, active GA-s were obtained with the same Rf-value as the free GA-s found in the acidic ethyl-acetate fraction.

From the linden seeds, we have isolated six kinds of free GA-s, among which four have been identified (GA_1, GA_4, GA_9, GA_{16}) and their succession according to concentration was defined. A definite difference exists in the endogenous gibberellin spectrum of the different types of seeds studied by us, but in every type the endogenous gibberellins showing the greatest biological activity and changing the most intensively during inductive treatment were found to be the GA_1 and the GA_4.

The diffusion method — also giving an idea of the GA transmembrane movement — was introduced to characterize the GA-supply of the tissues treated with the growth retardants.

It was proved that the results obtained with the antigibberellins (CCC, AMO—1618) cannot be applied for the purpose of judging the significance of the de novo GA-synthesis, either in the early phase of germination, or in the course of later development, since these retardants exhibit a complex effect on the metabolism of the GA-s: apart from inhibiting their biosynthesis they also have effect on the interconversions, they influence the ratio of the free and bound GA-s as well as the symplastic motion of the GA-s in the plant.

We have demonstrated that the GA-supply of the tissues treated with the growth retardant can be satisfactory even besides a free GA-level lower than the control, since the retardant enhances the GA movement in the plant.

We have concluded that the dormancy-maintaining effect of ABA, besides its direct effect of inhibiting embryo growth, can also be attributed to its role of hindering the release of the gibberellins from bound forms.

It has been proved by the studies on the changes during stratification of the endogenous ABA content, by model-experiments with excised embryos as well as by treatments of intact seeds with exogenous hormones that the ABA and other growth inhibitors play an important role in the maintenance of dormancy.

We have pointed out the significance of hormone-interactions and allosteric effects in the breaking of dormancy of the linden embryos and in the development of the germination ability. Dormancy release in the linden seeds is a process consti-

tuted of several phases in which the increase to optimal level of the promotor hormones (GA, IAA, cytokinin) and the optimal sequence of their appearance are of extreme importance, besides the decrease of the inhibitor levels caused by cold-effect. In this sequence, gibberellin is not a hormone inevitably standing in the first place in every seed; our data have otherwise refuted the generally decisive role of the GA-s played in the state of dormancy.

According to our results the dormancy of the *Tilia* seeds is firstly regulated from the direction of the inhibitors. Therefore, the role of the cold-effect is not considered to be the optimalization of the endogenous GA-level, but in the decrease in inhibitor-content and development of the ensuing new, favourable hormonal ratios.

Practical importance of the results

When selecting the topic of our study and setting the experimental targets, it was expected to gain such experiences in the course of characterizing the dormancy of the linden seeds and clarifying the hormonal regulation of dormancy, in the possession of which a proposal can be made for elaborating a method of fast germination without stratification, applicable also on a large scale. By means of comprehensive studies, however, it was proved that the characteristic anatomic structure and special nature of dormancy of the linden seeds/linden fruits do not make it possible to omit cold-stratification and have it substituted with chemical methods. This is so because the application of promotor exohormones for breaking of dormancy of the linden seeds is not successful without cold-effect.

The speeding up the germination of the linden seeds is possible by removing the fruit-coat hindering swelling and by the scarification of the seed-coat. These, however, are operations requiring much manual work; thus are only expediential for shortening the duration of experiments in case of laboratory studies. Therefore, our experimental data obtained with the linden seeds verify the practice of stratification.

Nevertheless, our results obtained upon treatment of the intact seeds with hormone combinations provide possibility for shortening the procumbent period.

The dissertation may provide useful data to the better understanding of the physiological processes taking place during vernalization, too; and our results regarding the mechanism of effect of the growth retardants as well as the interactions between the phytohormones can be utilized indirectly, in the practical use of the growth-regulating substances.

Selected publications relating to the dissertation

NAGY, M. and SZALAI, I. (1973): Dormancy in fruits of *Tilia platyphyllos* SCOP. I. — Acta Biol. Szeged. *19*, 71-77.

SZALAI, I. and NAGY, M. (1974): Dormancy in fruits of *Tilia Platyphyllos* SCOP. II. — The inhibitor substance content of dormant fruits. — Acta Bot. Acad. Sci. Hung. *20*, 389-394.

NAGY, M. (1975): Changes in the endogenous gibberellin content during stratification in *Tilia platyphyllos* SCOP. seeds. — 2nd Symp. Plant Growth Reg., Sofia, pp. 28.

NAGY, M. (1976): Dormancy in fruits of *Tilia platyphyllos* SCOP. III. A possible role of IAA and cytokinins in breaking dormancy. — Acta Biol. Szeged. *22*, 47-56.

NAGY, M. and GÖNDÖR, E. (1977): Change in the endogenous gibberellin content during swelling of *Lupinus albus* seeds. — Acta Biol. Szeged. *23*, 39-47.

NAGY, M. (1978): Free and bound gibberellins in *Tilia platyphyllos* SCOP. seeds. — 14-e Naucsno-koordinacioonoe szovescsenie i szimpozium po teme I—16. Szeged, pp. 19.

NAGY, M. (1980): Dormancy in fruits of *Tilia platyphyllos* SCOP. IV. Changes in the endogenous gibberellin content during stratification. — Acta Agron. Acad. Sci. Hung. *29*, 1-11.

NAGY, M., PATAKY, SZ. and KÉRI, Á. (1981): Dormancy in fruits of *Tilia platyphyllos* SCOP. V. Possible role of chilling stratification in breaking dormancy. — Acta Biol. Szeged. *27*, 127-137.

NAGY, M. and HODUR, C. (1982): Effect of a treatment with abscisic acid (ABA) on the changes of endogenous gibberellin content in *Phaseolus vulgaris* L. seeds during their swelling. — Acta Biol. Acad. Sci. Hung. *33*, 77-86.

NAGY, M. and TABI, ZS. (1982): Effect of CCC on the amount of diffusible gibberellins in bean plants. — Biochem. Phys. Pflanzen. *177*, 725-728.

NAGY, M., BUBÁN, T. and PATAKY, SZ. (1983): Effect of chilling stratification on nuclear nucleic acid content in the radicle of the embryos of *Tilia platyphyllos* SCOP. — Zeitschr. Pflanzenphysiol. *112*, 187-189.

NAGY, M. and HODUR, C. (1984): Effect of CCC on the amount of endogenous gibberellin content in *Phaseolus vulgaris* seeds during their swelling. — Acta Agron. Acad. Sci. Hung. *33*, 51-57.

NAGY, M. and KÉRI, Á. (1984): Role of the embryos in the cytolysis of the endosperm cells during the germination of the seeds of *Tilia platyphyllos* SCOP. — Biochem. Phys. Pflanzen, *179*, 145-148.

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SHORT COMMUNICATION

HIGHER ORGANIZED SPOROPOLLENIN BIOPOLYMER STRUCTURES AND THE EXPLOSION OF THE POLLEN GRAINS UNDER SCANNING EFFECT

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The first TEM pictures on the higher organized globular biopolymer units of the sporopollenin were first published in 1974 (KEDVES et al.). This structure was surprising in contrast to the fibrillar or lamellar concepts of the plant cell wall, and was criticized by ROWLEY and PRIJANTO (1977). Later helical substructures of the exine were described in several papers by ROWLEY et al., e.g.: 1981. SOUTHWORTH (1985) by acetolysis and hot 2-aminoethanol method re-discovered the granular sub-units of the exine of *Lilium longiflorum*, and established that "the granules are arranged in irregular pentagons", p. 1274. It was written that the exine consists of materials of three solubility and a new model of the exine substructure was published, where the system of polygons were surrounded by interconnected granules. During our new experimental studies with the *Helix* enzyme (KEDVES, 1986a,b) the globular biopolymer units were again described from the wall of recent *Corylus avellana* L. and fossil *Botryococcus braunii* KÜTZ. from the oil shale of Hungary. Later my new experiments with a modified method of 2-aminoethanol and $KMnO_4$ resulted in a very characteristic pentagonal polygon biopolymer structure (Fig. 1—3), on the exine of *Taxus baccata* L. The ultrastructure of the endexine of this species without degradation is characteristically lamellar. The higher organized biopolymer units are composed from globular base units, which are connected with short arms, cf. SOUTHWORTH (1985, 1986), well shown in Fig. 2. This structure is similar to a crystalline organisation. Probably this biopolymer organisation may explain the explosion of the pollen grains under "scanning effect" on high acceleration voltage. The acceleration of the pollen grains may deform this polygonal structures, and this is the cause of the explosion. In all probability, the arms will be broken and the collapse of the globular units liberate a relatively high energy. This is the reason why the place of the explosion, the gold-palladium and the adhesive have been evaporated (KEDVES 1986c, p. 208, Fig. 1). Since the biopolymer units of the wall of the *Botryococcus* algue from the oil shale are similar to those of the pollen exines, it may be hoped that with a rentable technology the oil shale can be a new energy basis, by the liberation of the binding energy of the wall biopolymer structure.

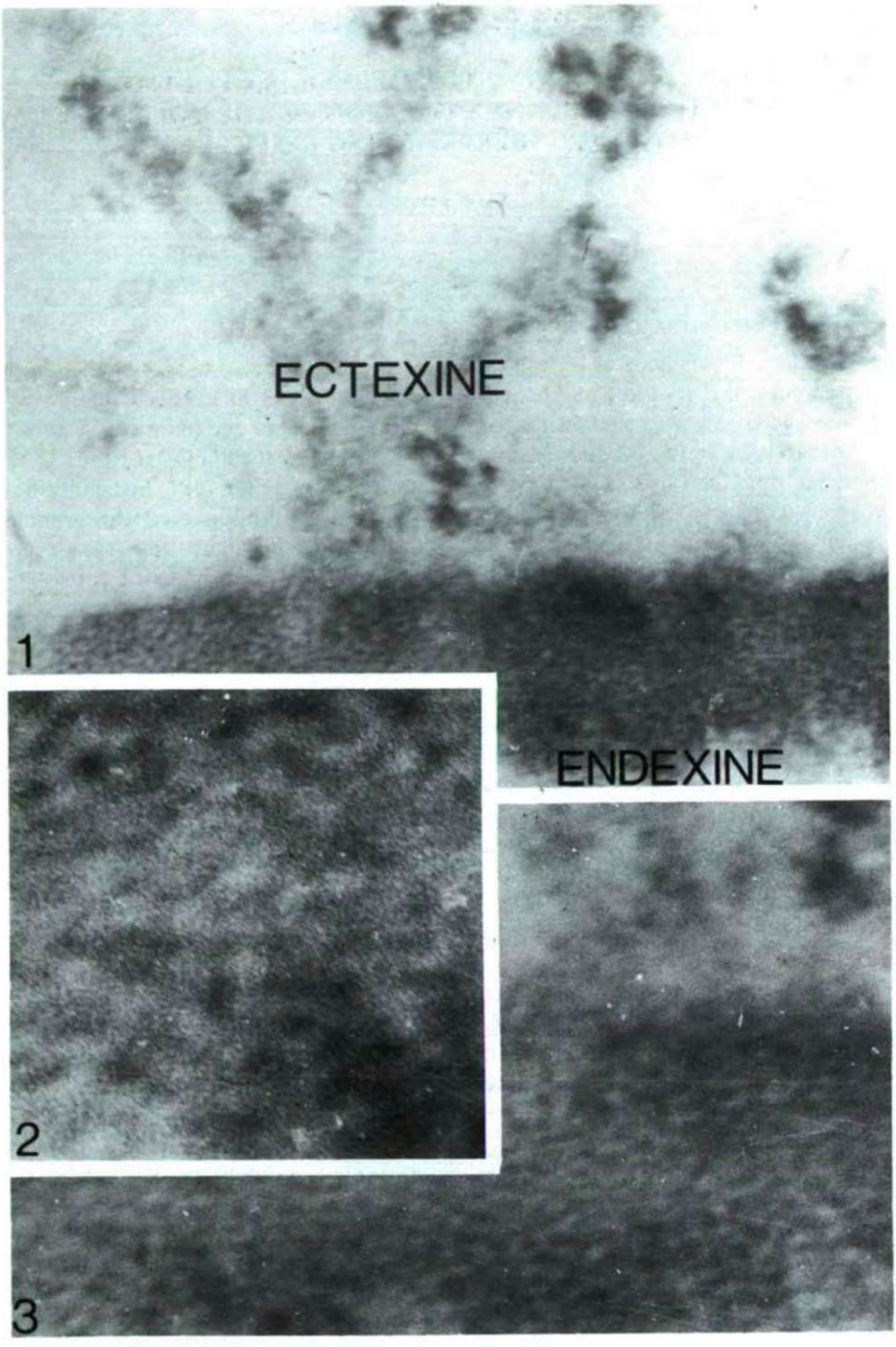
ECTEXINE
1
ENDEXINE
2
3

Fig. 1—3.
Taxus baccata L., partially degraded exine (20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 24 °C, length of time 24^h. + 10 ml $KMnO_4$ 1%, temperature 24 oC, length of time 12h).
1. x250000, 2. x1 million, 3. x500000.

References

KEDVES, M. (1986a): Dégradation expérimentale des colonies du genre *Botryococcus* des schistes pétrolifères du Tertiaire supérieur de Hongrie. — Acta Biol. Szeged. *32*, 39-48.

KEDVES, M. (1986b): In vitro destruction of the exine of recent palynomorphs I. — Acta Biol. Szeged. *32*, 49-60.

KEDVES, M. (1986c): Explosion of pollen grains under the electron beam effect of the scanning electron microscope. — Acta Biol. Szeged. *32*, 207-208.

KEDVES, M., STANLEY, E.A. and ROJIK, I. (1974): Observations nouvelles sur l'ectexine des pollens fossiles des Angiospermes de l'Éocène inférieur. — Pollen et Spores *16*, 425-437.

ROWLEY, J.R., DAHL, A.O., SENGUPTA, S. and ROWLEY, J.S. (1981): A model of exine substructure based on dissection of pollen and spore exines. — Palynology *5*, 105-152.

ROWLEY, J.R. and PRIJANTO, B. (1977): Selective destruction of the exine of pollen grains. — Geophytology *7*, 1-23.

SOUTHWORTH, D. (1985): Pollen exine substructure. I. *Lilium longiflorum*. — Amer. J. Bot. *72*, 1274-1283.

SOUTHWORTH, D. (1986): Substructural organization of pollen exines. — Pollen and Spores: Form and Function, 61-69.

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CHRONICLE

The following persons have deceased: Dr. GÁBOR FARKAS, academician and Dr. FALUDI, Mrs. ÁGNES DÁNIEL, honorary university professor at the Department of Plant Physiology, as well as Dr. MÁRIA MÉSZÁROS, retired lecturer.

Scientific degrees

The doctor's degree in biological sciences has been earned by Dr. GYULA L. FARKAS with the dissertation "The body development of 10—18 years old children and youth and the menarche-age of girls in South-Hungarian Plain".

The candidate's degree in biological sciences has been earned by Dr. ATTILA BARANYI with the dissertation "Elemental processes of learning and memory of cellular level in the cerebral cortex" and by Dr. MAGDOLNA SZENTHE with the dissertation "Characteristics of the paroxysmal action evoked by aminopyridin in the primer and mirror focus".

Appointments

The Cultural Minister has appointed Dr. ÉVA FEKETE lecturer at the Department of Zoology, and Dr. JÁNOS NEMCSÓK lecturer at the Department of Biochemistry.

Honours

Prof. Dr. T. HORTOBÁGYI, former head of the Department of Botany, became doctor of fifty years' standing.

Prof. Dr. M. KEDVES, research councilor (Department of Botany), held an inaugural lecture with the title "Dégradation experimentale de la paroi pollinique" at the VI. Symposium of the A.P.L.E., and was awarded the medal of the Salamanca University by Prof. Dr. D. PEDRO AMAT MUÑOZ, Rector, for carrying out high-

standard research activity as well as for the results achieved in the framework of the Hungarian-Spanish scientific collaboration (Fig.).

Medal of the Salamanca University

Varia

Prof. Dr. L. SZALAY, head of the Department of Biophysics, has been elected a member of the six-man board of officer of the European Society for Photobiology.

Dr. Gy. FARKAS, head of the Department of Anthropology, has been elected a member of the Executive Committee of the Hungarian National Section of the E.A.A. (European Anthropological Association).

The XIX. Congress of the Hungarian Biological Society was organized in Szeged. Chairman: Dr. L. ALFÖLDI, academician; vice-presidents: Prof. Dr. O FEHÉR and Dr. S. GULYÁS, lecturer; secretary: Dr. L. HALMÁGYI. 81 lectures were presented from the various fields of biology.

Publications

SZALAY, L. — RINGLER, A. (1985): Biophysics. — Educational Publishers, Budapest. The authors of the book won the Niveau-prize of the Ministry of Public Education.

The following works were published in the series Studia Biologica Academiae Scientiarum Hungariae:

KEDVES, M. (1986a): Introduction to the Palynology of pre-Quaternary deposits. Part I. — Publishing House of the Hungarian Academy of Sciences, Budapest.

KEDVES, M. (1986b): Introduction to the Palynology of pre-Quaternary deposits. Part II. — Publishing House of the Hungarian Academy of Sciences, Budapest.
KEDVES, M. (1986c): Paleogene fossil sporomorphs of the Bakony Mountains. Part IV. — Publishing House of the Hungarian Academy of Sciences, Budapest.

Academic membership

Prof. Dr. L. FERENCZY, head of the Department of Microbiology was elected in May 1987 as a corresponding member of the Hungarian Academy of Sciences.

Commission

The Department of Biological Sciences of the Hungarian Academy of Sciences has commissioned Professor FERENC ZSOLDOS, Head of the Department of Plant Physiology at Attila József University, to chair the Hungarian National Committee of the Federation of European Societies of Plant Physiology.

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BOOK REVIEW

KNOBLOCH, E. — MAI, D.H. (1986): Monographie der Früchte und Samen in der Kreide von Mitteleuropa. — Rozpravy Ustr. ust. geol. *47*, 219 pp., plates I—LVI, 52 figs. in the text.

May be ordered: ARTIA Aussenhandelsbetrieb, Ve Smečkách 30, 111 27 Praha 1, Tschechoslowakei, PF 790.

This monograph on the fossil fruits and seeds from the Cretaceous of Central Europe is based on the specimens of a collection of twenty years (1962—1982). The localities studied are as follows: Czechoslovak Socialist Republic (Peruc Member, Klikov Formation, the Coniacian and Santonian in northern Bohemia, flysch in the Moravian Carpathians); in the German Democratic Republic (Quedlinburg, Walbeck, Eisleben); in the flysch in Poland; in Hungary (Senonian in the Bakony Forest Mountains); in Austria (flysch around Vienna, Gosau Formation in Tyrol); in the Federal Republic of Germany (Cretaceous in northern Bavaria, Hergenrath Member in the vicinity of Aachen) and in the Netherlands (Hergenrath Member in southern Limburg). Macro-remnants from 114 localities were studied. All stages of the Upper Cretaceous (Cenomanian, Turonian, Coniacian, Santonian, Campanian Maestrichtian) were investigated

270 species from 85 genera were determined or described; 2 new genus and 162 new species. As botanical affinities the following taxa were established: *Taxodiaceae, Pinaceae, Zingiberaceae, Typhaceae, Magnoliaceae, Nymphaeaceae, Hamamelidaceae, Platanaceae, Ulmaceae, Moraceae, Urticaceae, Juglandaceae, Rhoipteleaceae, Theaceae, Pentaphylaceae, Saurauiaceae, Clethraceae, Ericaceae, Cyrillaceae, Droseraceae, Rutaceae, Sapindaceae, Sabiaceae, Mastixiaceae, Araliaceae, Aquifoliaceae and Icacinaceae.*

The results of this monograph are very important from paleobotanical point of view, too. Most of the plant microfossil data from Europe are from the *Normapolles* group with unknown or with discussed botanical affinities. Really, the fossil fruits and seeds are unique to solve several problems of the evolution of the angiosperms. A great part of the families from the Central European Cretaceous are of Laurasian origin. The remnant assemblage refer to evergreen subtropical and temperate vegetation in the Northern Hemisphere. Some families have questionable botanical

affinities, or with both Laurasian and west Gondwanan regions. It is necessary to emphasize the stratigraphic and paleophytogeographic importance of this work.

With this monograph we have a new basis and opportunity to create new paleobotanical and paleobiological synthesis in respect of the angiosperm evolution and the changes in the vegetation character of the Cretaceous — Tertiary boundary. The 56 plates with high quality photographs, several prepared by SEM techniques are a very important basic document in the determination of the fossil seeds and fruits of the Senonian.

This book is recommended for all researchers working on the vegetation history of the Upper Mesozoic and the basal Tertiary.

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